INSTRUMENT MANUAL

1450-932-10
June 2004

WALLAC MicroBeta® TriLux

and

WALLAC MicroBeta® JET

Liquid Scintillation and Luminescence Counters

PerkinElmer®
precisely.

Wallac 1450

MicroBeta® TriLux

and

MicroBeta® JET

Liquid Scintillation and Luminescence Counters

Valid for MicroBeta TriLux with software version 4.7 and MicroBeta JET with software version 5.2

PerkinElmer®
precisely.

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Warning

This equipment must be installed and used in accordance with the manufacturer's recommendations. Installation and service must be performed by personnel properly trained and authorized by PerkinElmer Life and Analytical Sciences.

Failure to follow these instructions may invalidate your warranty and/or impair the safe functioning of your equipment.

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Part 1 Introduction

1. Introduction

1.1 Guide to the Instrument manual

There are two versions of Wallac 1450 MicroBeta® from PerkinElmer Life and Analytical Sciences described in this manual: MicroBeta TriLux and MicroBeta JET. Most of the information is common to both models, where there is a difference it is noted. In Part 2 of this manual, alternative chapters with the number 2J.xx are included to describe JET features. The xx is the same number as the equivalent chapter for MicroBeta TriLux.

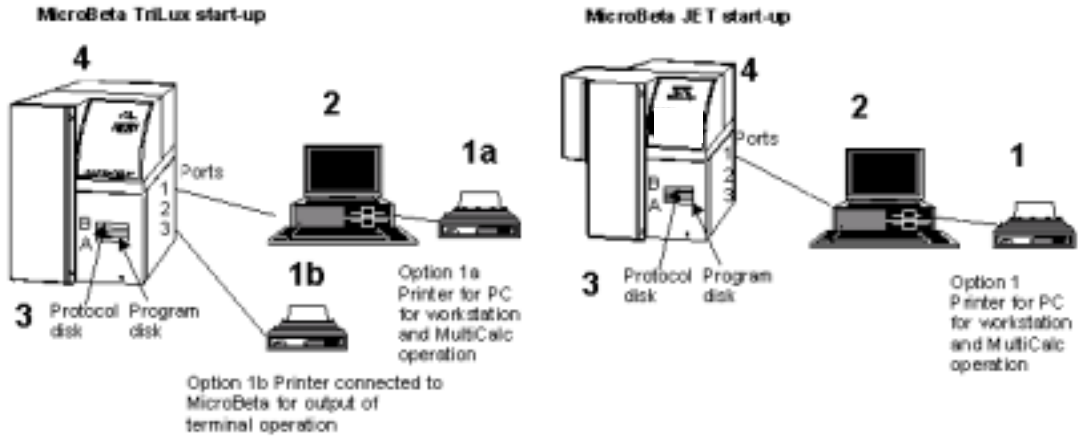
Normal start-up is described in section 1.2. On the following pages you will find compressed operating guides for MicroBeta TriLux and MicroBeta JET. Operations include references to the appropriate chapter in part 2 or part 2J of this manual where you will find a more detailed explanation. ***If you are using MicroBeta workstation software, refer to the separate User manual instead of section 2 in this manual. You only need to refer to section 2 if you work in terminal mode.***

Part 3 of the manual describes the instrument, including specifications. There is also chapter describing the routine maintenance to be done by the user.

Part 4 is only needed during installation.

Part 5 is the alphabetical index.

1.2 Instrument start-up



1. Switch on the printer (1a or 1b depending on your configuration), see the appropriate figure above.

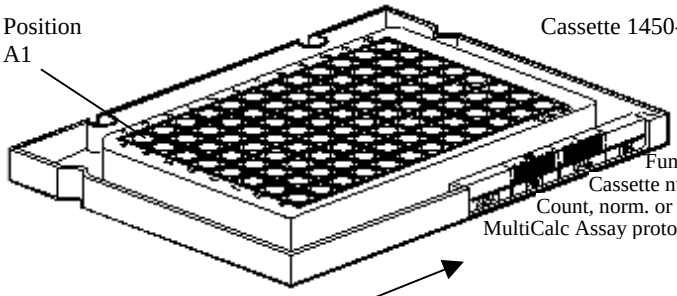
2. Switch on the PC and start up the terminal emulator program or start up MultiCalc and go to the terminal option by pressing F1 (=COUNTER) followed by F3 (=TERMINAL). See chapters 2.31 and 2.22 respectively. If you are using the workstation, start the program running as described in the separate User manual.

3. Insert the MicroBeta program disk into disk drive A, the lower disk drive of the counter, and the protocol disk into disk drive B, the upper disk drive.

4. Switch on MicroBeta with the power switch at the back of the counter. Loading takes some 3 minutes, then the counter is ready for operation.

Operating MicroBeta® TriLux

(See chapters in *italics* for more information on any subject)



Code labels (barcodes) are fixed to an ID-support plate which is clipped onto a cassette to show MicroBeta the counting protocol to use. *(2.4)*

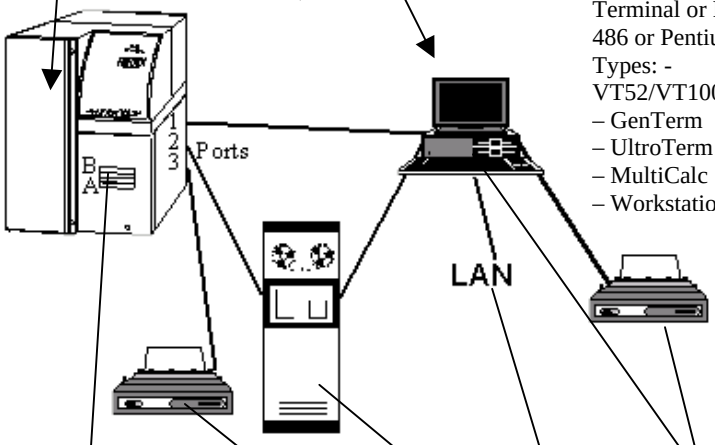
A **counting protocol** is a set of parameters e.g time, isotope, single/ dual label etc. which control counting. *(2.24, 2.6, 2.7 2.12, 2.14, 2.19, 2.33)*

Counting protocols use either **CPM normalization** *(2.23)* or **DPM standardization** *(2.12)* info. without or with crosstalk correction *(2.7)*. Normalization ensures the counting **efficiency** of each detector is the same. Standardization additionally calculates the amount of chemical **quenching**. Possible **crosstalk** between samples can be corrected for. Normalization and standardization each require counting a special set of samples. This will normally be done separately from routine operation. The results will be stored in MicroBeta and called into use in normal counting by means of the counting protocol.

1 Fix code labels to sample cassettes *(2.4)*
2 Load cassettes into MicroBeta. Close the cassette rack compartment door *(2.17)*
3 To start counting *(2.5)*:
Use the Windows Workstation
Alternatively press:
For MultiCalc: F1 (COUNTER) then Enter
For terminal etc: Protocol number or A

For interrupt or power failure info. see *(2.16)* and *(2.13)*

4 The results *(2.21, 2.26, 2.32)* are sent to selected output devices *(2.8, 2.29)*



You can use the workstation, a terminal, a terminal emulator or MultiCalc to control MicroBeta. The **workstation** is described in a separate user manual.
MultiCalc *(2.22, 2.31)* can be used to make assay protocols. An assay protocol tells which counting protocol MicroBeta is to use and what should be done with the data when it is obtained e.g. quality control analysis.
Terminal mode is available with MultiCalc and should be used if you want to edit normalizations, standardizations and counting protocols from within MultiCalc.
With a **terminal** *(2.31)* you can get results to your screen, print them out or store them on a disk in MicroBeta.
A **terminal emulator** *(2.31)* is a program which makes a PC act as a terminal. You can have results stored on your PC. You can also use your PC for other tasks during counting.

Results are sent to MicroBeta drive B during **terminal** operation. For diskette information see *(2.10)*

The printer connected to MicroBeta port 3 is used for **terminal** and **terminal emulation** printing.

Output to external computer e.g a **mainframe.** Connection to port 2 or PC.

Local area network connection (LAN) using the PC.

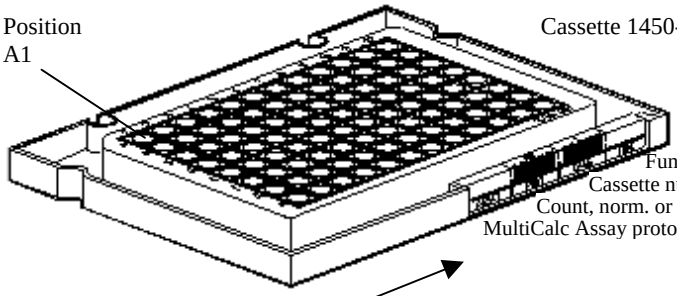
Results go to PC connected to port 1 in **terminal emulation** & **MultiCalc**. See *(2.10)* for diskette info. MultiCalc printout goes to the printer connected to the PC.

For **System setting** info. e.g. printer or terminal type *(2.30)*
For **Help** type H, h or ? *(2.15)*

Entering commands into MicroBeta is described in *(2.1)*
MultiCalc has its own manual.

Operating MicroBeta® JET

(See chapters in *italics* for more information on any subject)



Code labels (barcodes) are fixed to an ID-support plate which is clipped onto a cassette to show MicroBeta the counting protocol to use. *(2.4)*

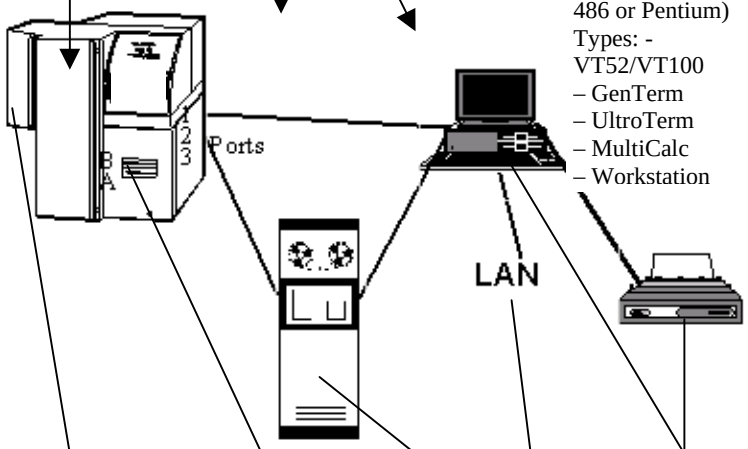
A **counting protocol** is a set of parameters e.g time, isotope, single/ dual label etc. which control counting. *(2.24, 2.6, 2.7 2.12, 2.14, 2.19, 2.33)*

1 Set up injectors *(2J.0)*
2 Fix code labels to sample cassettes *(2.4)*
3 Load cassettes into MicroBeta. Close the cassette rack compartment door (*2.17)*
4 To start counting *(2.5)*:
Use the Windows Workstation
Alternatively press:
For MultiCalc: F1 (COUNTER) then Enter
For terminal etc: Protocol number or A

For interrupt or power failure info. see *(2.16)* and *(2.13)*

5 The results *(2.21, 2.26, 2.32)* are sent to selected output devices *(2.8, 2.29)*

Counting protocols use either **CPM normalization** *(2.23)* or **DPM standardization** *(2.12)* info. without or with crosstalk correction *(2.7.)* Normalization ensures the counting **efficiency** of each detector is the same. Standardization additionally calculates the amount of chemical **quenching**. Possible **crosstalk** between samples can be corrected for. Normalization and standardization each require counting a special set of samples. This will normally be done separately from routine operation. The results will be stored in MicroBeta and called into use in normal counting by means of the counting protocol.



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MultiCalc *(2.22, 2.31)* can be used to make assay protocols. An assay protocol tells which counting protocol MicroBeta is to use and what should be done with the data when it is obtained e.g. quality control analysis.
Terminal mode is available with MultiCalc and should be used if you want to edit normalizations, standardizations and counting protocols from within MultiCalc.
With a **terminal** *(2.31)* you can get results to your screen, print them out or store them on a disk in MicroBeta.
A **terminal emulator** *(2.31)* is a program which makes a PC act as a terminal. You can have results stored on your PC. You can also use your PC for other tasks during counting.

Injector assembly fits here with the module(s) below. Port 3 is used for connection to the injector modules. Settings: Baud 6, Parity 1, Data bits 8, Stop bits 2, Handshake 1, see *(2J.30)*

Results are sent to MicroBeta drive B during **terminal** operation. For diskette information see *(2.10)*

Output to ext. computer e.g a **mainframe.** Connect to port 2 or PC.

Local area network connection (LAN) using PC.

Results go to PC connected to port 1 in **terminal emulation** & **MultiCalc**. See *(2.10)* for diskette info. MultiCalc printout goes to printer connected to the PC.

For **System setting** info. e.g. printer or terminal type *(2.30)*
For **Help** type H, h or ? *(2.15)*

Entering commands into MicroBeta is described in *(2.1)*
MultiCalc has its own manual.

Part 2 Operating information

This part has information common to both MicroBeta TriLux and MicroBeta JET and also information only valid for MicroBeta TriLux. In the latter case there are parallel chapters at the end of this part which are valid only for MicroBeta JET. These chapters are given the same number as the MicroBeta TriLux chapter but with a J after the 2 e.g. 2J.1 is the chapter giving MicroBeta JET information corresponding to the MicroBeta TriLux information in chapter 2.1. There is information at the head of each MicroBeta TriLux chapter referring you to the relevant MicroBeta JET chapter.

2.1 Beginning operation of MicroBeta TriLux

(See chapter 2J.1 for JET information)

The following conventions are followed all through the MicroBeta user interface:

2.1.1 Menu selections

Menu selections can be entered when the system is in one of the following states:

```
Ready>                  = Ready for next operation
Protocols>              = Protocol operations
Count>                  = Counting operations
Info>                   = Additional info.
System>                 = System level operations
Counting protocol>      = Counting protocol editor
CPM normaliz. protocol> = CPM norm. protocol editor
DPM standard. protocol> = DPM std. Protocol editor
Assay protocol>         = MultiCalc assay protocol operation
Conveyor>               = Conveyor operation
Total count rate>       = Total count rate manipulation
```

Command options available, e.g. those shown below, are displayed before the prompt text, e.g. Ready:

```
(H)elp          (I)nfo
(C)ount         (P)rotocols     (S)ystem

Ready>
```

The command is executed by pressing the letter (either lower or upper case) enclosed inside parentheses. However, commands that include a protocol number (e.g. giving a pure protocol number in the Ready state to start counting of that protocol) must be completed by pressing the Enter key.

Help text can be obtained by pressing H, h or ?. Exit to a higher level by pressing Q. Pressing Control-E (the Ctrl and the E keys at the same time) exits always to the Ready state.

2.1.2 Confirming questions and messages

Quite often MicroBeta displays a message such as:

```
Press any key when ready or / to exit ->
```

This kind of message is used to give you time to think if this is really the correct operation and to check that everything is prepared for the operation. Press any key (such as the space bar) when ready to continue. Otherwise you can cancel the operation by pressing slash /.

Messages such as:

```
Do you accept the new terminal setting? (Y/N) ->
```

are used to notify you that the operation under question can affect the functioning of the instrument, counting results etc. Press Y=Yes to accept the operation or N=No to cancel it.

2.1.3 Input of data

The rest of the user interface consists mostly of data input. Data input differs somewhat from menu selection and confirming questions.

- The default or the current value is usually displayed before an arrow sign ->.

- After typing the new value you must press Enter.

- Press the Enter key alone to leave the default or current value unchanged.

- To get help about the subject under question press Enter after H, h or ?.

- Press slash "/" or backslash "\" to exit input mode. In the protocol editor "/" exits with saving the protocol and "\" without saving it. Elsewhere usually both cause exit without saving the data.

- Input data can be edited (see below).

2.1.4 Editing input data

If you make a mistake when entering a new input value, you can use the Backspace key (sometimes labelled as BS or a thick left arrow) or the DEL-key to erase the previous character. If the input data is quite long this can be tedious. For this kind of situation a simple keyboard editing facility is provided.

Keyboard editing only uses a few keys so it is easy to learn. The default value or the current value of input data is used as a template for entering a new value. So if you notice that you have made a mistake during an early phase of entering a long input (such as output selection in

the protocol editor) you can press Enter to complete entering a new value and then go back to the same line to correct the mistake.

The editing keys are:

<- or Control-B Go back one character in the template (default or current value).

-> or Control-N Go to the next character in the template.

E.g. Control-B is entered by first pressing down the Control key (usually labelled with Ctrl) and then typing letter B (lower case or upper case does not matter). If you keep pressing both the Ctrl-key and letter B down for a while then the repeat function of the terminal sends several Control-Bs to MicroBeta.

Note that Control-B and Control-N move only inside the default or the current value (displayed before ->), not inside a newly typed text.

The cursor control keys (left and right arrows) can sometimes be used instead of Control-B and Control-N. This does not work with every terminal emulator. The up and down arrow keys can be used when moving up and down in the protocol editor or you can type Lx to jump to line number x. Please note that the Enter key must be pressed if making changes to any row.

As an example of keyboard editing, suppose you have entered the following printout selection (the second line):

```
Printer output: POS CTIME CCPM1 CCPM1%
-> POS CTIMR COUNTS1 CCPM1 CCPM1%
```

After you press Enter you will see the text:

```
Error in output heading : CTIMR
```

and an arrow -> will appear. Press the Control-N keys. As you do this, you will see the letters POS CTIMR COUNTS1 etc. appearing. After CCPM1% has been displayed the program will beep to inform that the end of the current value has been reached. Keep Control-B pressed down and you will see that characters at the end of output selection start disappearing. Continue until the cursor blinks just after CTIMR (_ shows the place of the cursor):

```
-> POS CTIMR_
```

If you go too far then stop pressing Control-B and press Control-N to make the lost characters visible again. When the cursor is placed just after CTIMR (as in the example) press Backspace or the DEL key once and R will disappear. Type the letter E.

```
-> POS CTIM_
```
(Backspace or DEL pressed)

```
-> POS CTIME_
```
(Letter E typed)

Keep pressing Control-N until there is a beep and you get:

```
-> POS CTIME COUNTS1 CCPM1 CCPM1%
```

You can now continue typing new headings after CCPM1%. E.g.:

```
-> POS CTIME COUNTS1 CCPM1 CCPM1% MEAN1 CV1%
```

Now press Enter to complete editing.

If you later want to remove the heading COUNTS1, go to line 31 to get the programmable output selection. Keep pressing Control-N until you are just past COUNTS1:

```
-> POS CTIME COUNTS1_
```

Press Backspace or DEL 8 times so that COUNTS1 and the preceding space disappears:

```
-> POS CTIME_
```

Keep pressing Control-N until there is a beep and press Enter:

```
-> POS CTIME CCPM1 CCPM1% MEAN1 CV1%_
```

You can also add a new heading afterwards. Go to programmable output selection again. Keep pressing Control-N until the cursor is just after CTIME:

```
-> POS CTIME_
```

Now type space and the text SQP(I):

```
-> POS CTIME SQP(I)_
```

Keep pressing Control-N until the program beeps and then press Enter:

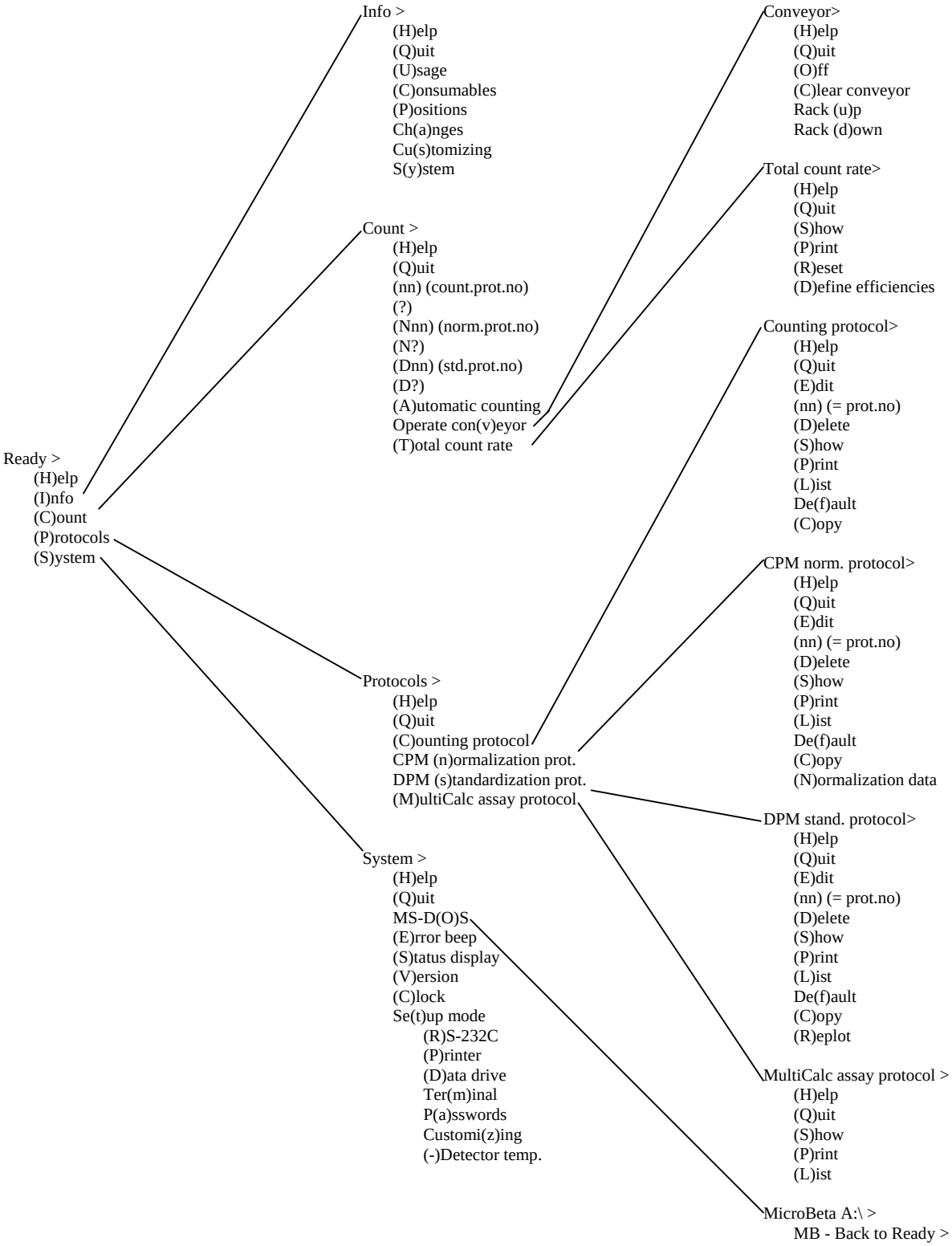
```
-> POS CTIME SQP(I) CCPM1 CCPM1% MEAN1 CV1%_
```

Note: Keyboard editing works with most of the type (2.1.3) data input

2.1.5 System operation

The figure following is the flow diagram of the whole MicroBeta operating system. It shows how the control letters inside parentheses lead to various functions. The functions are described briefly on the following pages.

Flow chart of MicroBeta TriLux commands (normal level)



MicroBeta TriLux commands (normal level) with a brief description of each

Operations in the Ready State

(H)elp	- Display Help text
(I)nfo	- Get information on using the instrument
(C)ount	- Start counting or operate conveyor
(P)rotocols	- Edit counting parameters
(S)ystem	- Edit system parameters

Count state operations are available in the Ready state too.

Operations in the Info State

(H)elp	- Display Help text
(Q)uit	- Back to the Ready state
(U)sage	- View a short user's manual
(C)onsumables	- View a list of consumables available
(P)ositions	- View normalization and standardization positions
Ch(a)nges	- View a list of changes after the previous version
Cu(s)tomizing	- View information on how to customize the program
S(y)stem	- View information about the system

Operations in the Count State

(H)elp	- Display Help text
(Q)uit	- Back to the Ready state
(nn) (count.prot.no)	- Start automatic counting by giving protocol number
(?)	- Start automatic counting by selecting protocol from list
(Nnn) (norm.prot.no)	- Start CPM normalization by giving protocol number
(N?)	- Start CPM normalization by selecting protocol from list
(Dnn) (std.prot.no)	- Start DPM standardization by giving protocol number
(D?)	- Start DPM standardization by selecting protocol from list
(A)utomatic counting	- Start automatic counting using IDs
Operate con(v)eyor	- Conveyor operations
(T)otal count rate	- Show and reset total counts
(M)anual counting	- Start manual counting (only at Test level)

Operations in the Protocols State

(H)elp	- Display Help text
(Q)uit	- Back to the Ready state
(C)ounting protocol	- Edit counting protocol
CPM (n)ormalization prot.	- Edit CPM normalization protocol
DPM (s)tandardization prot.	- Edit DPM standardization protocol
(M)ultiCalc assay protocol	- Show and print MultiCalc assay protocol

Operations in the Counting Protocol State

(H)elp	- Display Help text
(Q)uit	- Back to the Ready state
(E)dit	- Edit or create and edit a protocol
(nn) (= prot.no)	- Edit a protocol by giving protocol number
(D)elete	- Delete a protocol
(S)how	- Show protocol parameters
(P)rint	- Print a protocol
(L)ist	- Print list of protocols
De(f)ault	- Give default values to a protocol
(C)opy	- Copy one protocol to another

Operations in the CPM Normalization Protocol State

The same operations as in the Counting protocol state, plus:

(N)ormalization data	- Print normalization data

Operations in the DPM Standardization Protocol State

The same operations as in the Counting protocol state, plus:

(R)eplot	- Plot DPM standardization curve

Operations in the MultiCalc Assay Protocol State

Contains only operations (S)how, (P)rint and (L)ist.

Operations in the Conveyor State

(H)elp	- Display Help text
(Q)uit	- Back to the Ready state
(O)ff	- Stop conveyor
(C)lear conveyor	- Move cassette to rack and move rack to the middle
Rack (u)p	- Move the rack up one level
Rack (d)own	- Move the rack down one level

Test level contains also:

Remove ca(s)s	- Move cassette to rack
(N)ext cass	- Move next cassette to conveyor and read ID
(P)rev cass	- Move previous cassette to conveyor and read ID
Cu(r)rent cass	- Move current cassette to conveyor and read ID
Ne(x)t pos	- Move cassette to the next position
(G)oto pos	- Move cassette to a specified position
C(l)ose detector	- Close detector block
Op(e)n detector	- Open detector block
Next le(v)el	- Move rack down to next measurement level

Reset r(a)ck	- Move rack to the highest level
(I)nit mask	- Initialize detector mask (small holes)
Change (m)ask	- Change detector mask
(T)est IDs	- Read and print ID of each cassette continuously
(F)ocus ID reader	- Focus and stop with ID reader on
(J)ust read IDs	- Read IDs continuously and show IDs on the screen
Print ever(y) ID	- Toggle printing of ID bitmaps in ordinary form
Print ra(w) IDs	- Toggle printing of ID bitmaps in raw form
Print (b)ad IDs	- Toggle printing of bad ID bitmaps in raw and ordinary form
Si(z)e checkpoint	- Move cassette to cassette type checkpoint
Calib (0)-pos	- Move cassette between zero point and D06
Calib det bloc(k)	- Move detector block up and down at mask change position
(.)Park det	- Park detector block for shipping
Shelf (1)..(16)	- Move rack to the specified shelf
(*)Calib sensors	- Calibrate sensor offset steps

Operations in the Total Count Rate State

(H)elp	- Display Help text
(Q)uit	- Back to the Ready state
(S)how	- Show the accumulated total count rate and activity
(P)rint	- Print the accumulated total count rate and activity
(R)eset	- Reset the accumulated total count rate and activity
(D)efine efficiencies	- Define counting efficiencies of the isotopes

Operations in the System State

(H)elp	- Display Help text
(Q)uit	- Back to the Ready state
MS-D(O)S	- Exit to DOS
(E)rror beep	- Set error sound on or off
(S)tatus display	- Set status display on or off
(V)ersion	- Show version number of program and MS-DOS
(C)lock	- Set date and time
Se(t)up mode	- Select Setup mode

Setup mode contains also:

(R)S-232C	- Set serial port parameters
(P)rinter	- Set printer parameters
(D)ata drive	- Select drive for data saving (results)
Ter(m)inal	- Set terminal parameters

P(a)sswords	- Print protocol passwords
Customi(z)ing	- Set environment strings to customize the program
(-)Detector temp	- Set thermostat (optional)

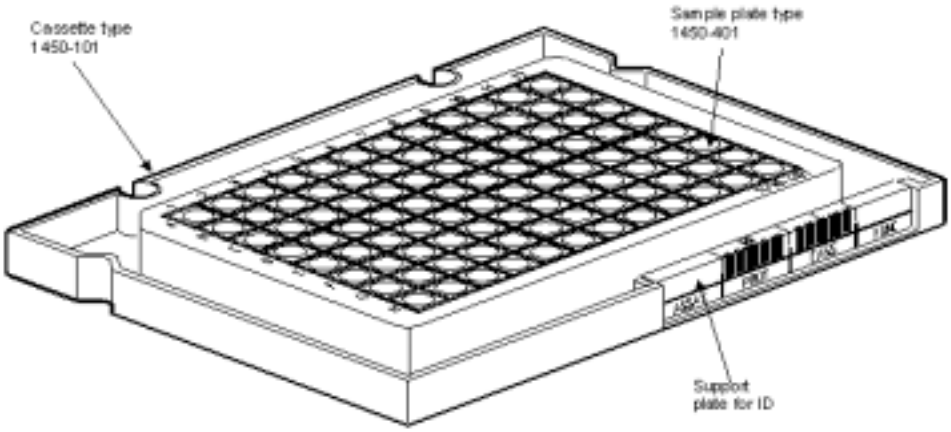
Test level contains also:

Serial (n)o	- Set instrument serial number
Temp cali(b)	- Edit temperature calibration values
Detector/shelf (u)sage	- Set detector usage during counting, set program support for the ParaLux board and set program support for the plate ID reader if installed and specify 1/16/32 shelf model

2.2 Cassettes

2.2.1 Construction

A MicroBeta cassette is designed to hold a microplate and to allow it to be moved from the rack to the conveyor and back again. There are three types of cassette that can be used in MicroBeta, a 96-well, a 24-well and a 384-well cassette. If the instrument has six or less detectors it can take either a 96 or 24 well cassette or a 96 or 384-well cassette depending on the mask used. If it has twelve detectors then only 96-well and 384-well cassettes can be used.



Cassette 1450-101. This has 96 sample holes with a diameter of 7.8mm. The cassette is used when counting 1450-401 (or equivalent) 96 well sample plates.

Cassette 1450-102. This has 24 sample holes with a diameter of 13.2 mm (see the figure). This cassette is used when counting 1450-402 (or equivalent) 24-well sample plates.

Cassette 1450-103. This has 96 sample holes. The cassette is used when counting 1450-407 plates.

Cassette 1450-104. This is a two piece cassette with 96 sample holes. This cassette is used when counting filtermats. The filtermat in a sample bag is placed between the base plate and the cover plate.

Cassette 1450-105. This has 96 sample holes. This cassette is used when counting 1450-410, 1450-405, 1450-419 or equivalent sample plates.

Cassette 1450-106. This has 96 sample holes. This cassette is used when counting e.g. Millipore MultiScreen™ Filtration plates or equivalent sample plates.

Cassette 1450-107. This is a two piece cassette with 24 sample holes. The cassette is used when counting Skatron filtermats cut into four pieces. The filtermat in a sample bag is placed between the two plates of the cassette.

Cassette 1450-110. This is an open-based cassette. The cassette is used when counting 24-well culture plates, e.g. Costar plates (or equivalent).

Cassette 1450-116. This is a two piece cassette with 24 sample holes. This cassette is used when counting filtermats. The filtermat in a sample bag is placed between the base plate and the cover plate.

Cassette 1450-117. This is a two piece cassette with 24 sample holes. This cassette is used when counting 4 mL sample vials. The vials are placed between the base plate and the cover plate.

Cassette 1450-118. This is a two piece cassette with 96 sample holes. This cassette is used when counting e.g. ^{32}P-labelled dot blot samples from filtermats such as 1450-423 Nylon membrane. No scintillator needs to be added to the filtermat allowing reprobing assays. The filtermat in a sample bag is placed between the base plate and the cover plate having solid scintillator in sample positions.

Cassette 1450-130. This has 384 sample holes. This is used when counting 384-well sample plates.

All the cassettes are made of white plastic with an optical surface in the sample holes.

2.2.2 Use

The sample plates are placed on top of the cassettes; the filters between the plates; the cassette can be labelled with an ID code. This is described in chapter 2.4 "Coding the cassette". Cassettes are loaded into MicroBeta as described in chapter 2.17 "Loading the cassette rack".

A cassette type is recognised automatically, i.e. whether it is a 384, 96 or 24 position cassette that is being counted. See chapter 2.9 for information about which positions detectors are in when counting samples in different types of plates.

2.2.3 Adapters and inserts

Adapter 1450-108. These adapters are used when counting Eppendorf tubes (or equivalent). The adapters are place on a 1450-102 cassette, a bottom tape is used if needed.

Inserts 1450-109. These inserts are used when counting 24 well culture plates. The inserts are placed in the sample wells in order to reduce crosstalk between the wells.

2.3 Clock setting

2.3.1 Current date and time

The current date and time are shown in the upper right corner of the screen during counting.

2.3.2 Adjusting the clock

When the instrument is installed you need to set the clock. Apart from this the clock will not normally need resetting as it has a battery back-up. However if you do need to adjust the clock proceed as follows.

Press S (System) in the Ready mode, then press C (Clock) to make changes to the clock. Enter the date and time in the format described below.

First the program asks for the date. Type it in using the format dd-mmm-yyyy where:

dd = day of the month (1-31)

mmm = month (the first three letters of the month in English i.e JAN, FEB, MAR, APR, MAY, JUN, JUL, AUG, SEP, OCT, NOV, DEC.)

yyyy = year (four digit year number)

E.g. 10-MAY-2000

When you have typed the date press Enter.

Note: You can also give the date in the format Year-month-day if preferred, e.g. 2000-5-10

Then the program asks for the time. Type it in using the format hh:mm:ss where:

hh = hours (0 - 23, 24 hour clock)

mm = minutes (0 - 59)

ss= seconds (0-59) (optional)

E.g. 14:57. When you have done this then press Enter.

The last line asks: Set clock. When Y (yes) is pressed the clock is started. To get back to the Ready state press Q (quit) to quit the System mode.

For more information on the System mode see chapter 2.30 System.

2.3.3 Counting time

During counting both the elapsed time from the beginning of counting and the set counting time are displayed on the screen (if the display is selected). If the number of cassettes is defined the estimated end time is also displayed.

2.4 Coding cassettes and filtermats

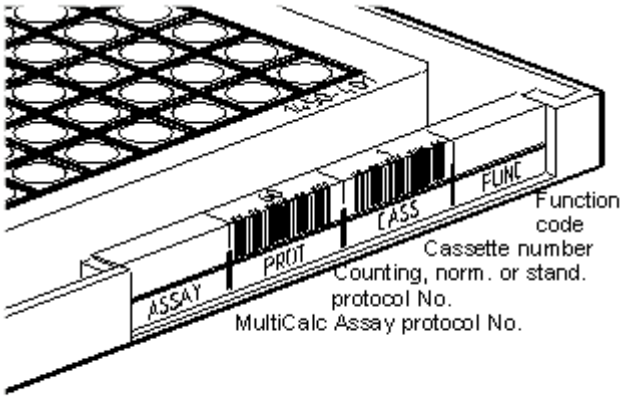
2.4.1 Cassette code system

Barcode labels are used for cassette and sample recognition. They are attached to the marked area of the ID support plate. This is a separate piece of plastic which can be clipped onto the appropriate area of a cassette as shown in the figure. When fixing ID labels on the ID support plate take care that the fixing area is clean.

Sometimes it happens that the protocol ID label is dirty or placed the wrong way round and the barcode reader fails to read it. In this case the default protocol (prot.No. 0) is used.

2.4.2 Cassette codes used

An ID support plate has four fields (areas) to which a barcode can be fixed. The meaning of each field is described below. The figure shows a counting cassette. The cassette number is 1 and the counting protocol to be used is 60.



ID support plate and barcodes

2.4.2.1 Function codes

The FUNC field can be labelled as follows:

No label - The cassette is a normal sample cassette without any special function

STD - The cassette is to be used for DPM standardization (See chapter 2.12 DPM Standardization) The number of the standardization protocol is given in the PROT field.

NORM - The cassette is to be used for CPM normalization (See chapter 2.21 CPM Normalization) The number of the normalization protocol is given in the PROT field.

STOP - counting will stop after this cassette has been counted.

2.4.2.2 Cassette number

This is a simple number in the range 0-99 and can be output with the results. If the function code is also a number then 100*cassette number + function code is output (in the range 0..9999).

2.4.2.3 Protocol number

This is normally a simple number in the range 0-99 (for the exception see Stop cassette below). The type of protocol it refers to depends on what is specified in the FUNC field. If nothing is specified in the FUNC field then it is the number of a counting protocol.

2.4.2.4 Assay protocol number

This field is for MultiCalc assay protocols only. Note that MultiCalc must be running when using this field, otherwise the results will be ignored.

If counting is started from MultiCalc but there is no Assay code label, then the cassette will be counted according to the other three fields but results will not be returned to MultiCalc. This can be used if you want to run standardizations or normalizations. You could, for example, have a normalization cassette counted first, followed by a cassette (or cassettes) labelled with an assay protocol. The counting protocol included in the assay protocol could make use of the normalization results just obtained. See chapters 2.20 and 2.22 for more information about protocols.

2.4.3 Stop cassette

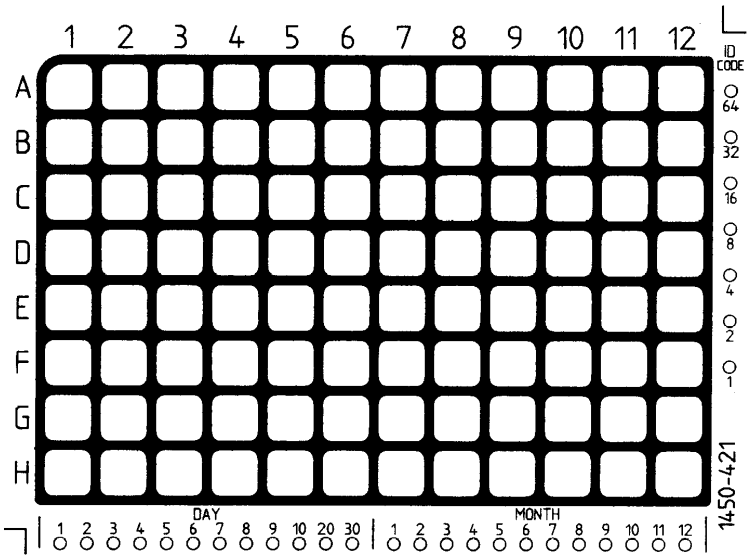
If you fix a STOP code to the last cassette it means that this cassette will be counted also. However it can be tedious to remove this STOP code and stick it to another cassette when more cassettes are loaded. To avoid this a stop cassette can be used. Stick a STOP code on both the protocol and function code field of an empty cassette and put that cassette after the last cassette to be counted.

2.4.4 Cassette operation

If MicroBeta is loaded with many sample cassettes that are to be counted with different protocols, the cassettes are coded with the respective protocol number. If there is no protocol number on the cassette, the cassette is counted with the same protocol as the previous one i.e. it

is considered to be part of the same assay. To stop the counting automatically the STOP code should be used.

2.4.5 Filtermat coding



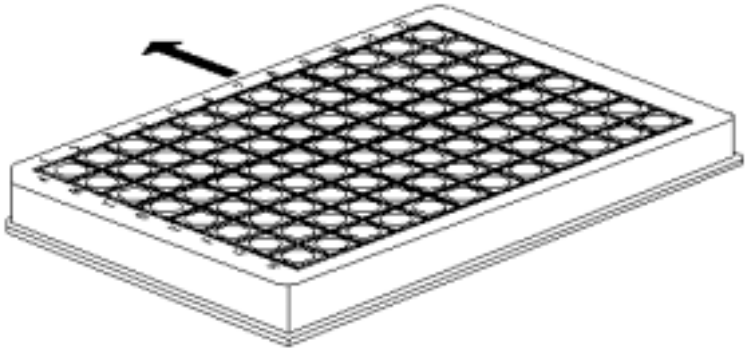
On the lower edge of the filtermat there are two groups of twelve small circles (see the figure). They are for marking the date. This is done by cutting or clipping off appropriate circles. The first twelve circles are to specify the day and the second twelve the month. In the figure above, the leftmost 9 circles stand for the numbers from 1 to 9 and the next three circles for 10, 20 and 30 respectively. E.g. the 25th day is marked by cutting off the 20 circle (the 11th circle from the left) and the 5 circle.

The month is marked using the rightmost 12 circles, the first circle corresponding to January, the second February etc.

The row of 7 small circles on the right-hand edge (next to sample column A12 to H12) is used for sample identification. The circles stand for the sequence 1, 2, 4, 8, 16, 32, 64. By cutting off appropriate combinations of circles the filtermat can be marked with a number in the range 1 to 127.

2.4.6 Plate ID reader

If the optional plate ID reader is installed then it is possible to read barcode IDs from plates. The following barcodes are supported: Codabar, Code 39, Code 128, Interleaved 2 of 5, UPL and EAN. The ID should be fixed to the upper part of the right side.



2.5 Counting

2.5.1 Starting counting

First load samples as described in chapter 2.17 Loading the cassette rack. You can then either start counting as a terminal operation (with a terminal, terminal emulator or terminal emulation in MultiCalc) or as a MultiCalc operation. In terminal operation the counter program must be in the Ready or Count state before you give the instruction to start counting. The options are as follows:

2.5.1.1 Automatic counting (terminal operation)

If your cassettes have protocol ID labels, press A in the Ready or Count state. The counting protocol to be used will be determined by the ID labels.

If your cassettes do not have ID labels, simply type the number of the protocol to be used and press Enter. The number you enter must be within the allowed range, 0-99. Pressing ? brings a list of protocols to select from.

If counting was started with an A-command and the first cassette has no counting protocol code, then the first assay will be counted using the default counting protocol (protocol number 0).

Any other cassette that does not have a counting protocol number will be counted with the protocol defined by the most recent cassette with a counting protocol number. This means that within one assay you only need to label the first cassette. A new label is only needed to start a new assay.

2.5.1.2 Automatic normalization or standardization (terminal operation)

If your cassettes have normalization (or standardization) protocol and function ID labels, press A. The counting protocol to be used will be determined by the ID labels.

If your cassettes do not have ID labels, type the protocol number preceded by N in normalization (e.g. N15) and D in DPM standardization (e.g. D15).

Typing N? or D? brings a list of normalization or standardization protocols to select from.

Normalization or standardization can be done before sample counting (See the chapters 2.12 and 2.21).

2.5.1.3 Automatic counting (MultiCalc)

Your cassettes must have Assay ID labels on them. Starting from the main MultiCalc menu press F1 (=COUNTER) and then press Enter to start counting. The Assay protocol to be used will be determined by the ID label.

If an Assay code is missing but there is a protocol code, the samples will be counted using the counting protocol defined but results will not be returned to MultiCalc.

If counting was started from MultiCalc but the first cassette has no protocol code, then the first assay will be counted using the default counting protocol (prot. No. 0) but results will not be returned to MultiCalc.

Any other cassette that does not have an Assay protocol number will be counted with the assay protocol defined by the most recent cassette with an assay protocol number. This means that within one assay you only need to label the first cassette. A new label is only needed to start a new assay.

2.5.1.4 Shelf number

The program will then ask for the shelf number of the first cassette. Give this number. The default is shelf number 1.

2.5.1.5 Delayed start

Counting can also be started after a delay. This is useful for e.g. incubation. To do this press D when starting counting. You must then enter the delay time in minutes and press Enter. The program will display the remaining time before starting the counting. Counting can be started immediately by pressing S or the operation can be cancelled by pressing Q.

```
Delay time before start (m) 60 ->
(S)tart counting (Q)uit
Time remaining: 59:58
```

2.5.2 During counting

The door of the counter should be properly closed during counting and it should not normally be opened unless counting has been interrupted by pressing O. If you need to add more cassettes then you can open the door and place the cassettes in a suitable empty place. Close the door. Counting of those samples which were in the counting position when the door was opened will be done again.

While counting is taking place the uncorrected CPM or CPS values are shown on the display of the Terminal PC. These are replaced by CCPM1, DPM1 or LCPS values when the whole plate

has been counted. In addition there is the information from the barcodes on the cassette: assay number, protocol number, cassette number, function codes and the number of the shelf from which the cassette was taken. There is also the current date and time for the assay, the counting time set in the protocol and the current counting time in seconds for the samples in the measuring position. If there is a specified number of samples (given on line 22 in the protocol), or normalization or standardization samples, the assay end time and date will be given also.

During counting of an Assay protocol in MultiCalc live information from the counter is not normally shown. However by pressing the F4 key (LIVE) some information will be displayed e.g. protocol No., current and set counting times, and positions that are counted

2.5.3 Stopping counting

Counting can be stopped anytime by just pressing O (off). The program terminates counting. If N (Next pos) or E (Next protocol) is pressed, the program starts to count the next sample position or the next assay respectively. If quick view is used then N stands for the next plate.

Counting of an Assay protocol in MultiCalc is stopped by pressing the F1-key. F2 and F3 are used for counting the next position or the next assay respectively.

2.6 Counting control

(See chapter 2J.6 for JET information)

2.6.1 Counting parameters

The counting process is controlled by the following parameters in the counting protocol:

```
10 Counting time
12 Precision (2 sigma)
41 Counting control
         Number of repeats
         Number of replicates
         Number of cycles
Maximum channel counts (a hidden parameter).
48 Delay between plates
```

Note: Assay protocol parameters are described in chapter 2.20 MultiCalc.

A CPM normalization protocol or DPM standardization protocol does not include parameter 41 Counting control. Instead the crosstalk normalization and crosstalk standardization protocols contain counting time for both crosstalk samples and standards, e.g.:

```
10 Counting time for crosstalk
11 Counting time for standards
```

2.6.2 Counting time

Counting will be stopped after the specified counting time (in seconds) has elapsed from the start of the counting, unless some other conditions have already stopped it. The maximum counting time is 999 999.9 s (more than 11 days).

2.6.3 Precision (2 sigma)

The statistical uncertainty in samples is expressed as the standard deviation or 'sigma' value and is calculated by dividing number one by the square root of the counting value. E.g. 10000 counts gives a 'one sigma %' value of 1%. The 2 sigma % value is two times the one sigma % value, or 2% for 10000 counts.

In MicroBeta, precision is given as 2 sigma % in a range from 0 to 99.9. Zero means that no precision test is used.

Make sure that the counting time is set long enough if precision is wanted. E.g. two samples of 1000 and 10000 CPMs will take 10 min and 1 min respectively to achieve a 2 sigma % value of 2%. Precision is checked once a second. The termination flag in results output will be set to

'PREC' if the precision has been reached. The counting stops if the precision has been reached in all detectors.

2.6.4 Number of repeats

To check sample stability or to check instrument performance, the same sample can be counted repeatedly, up to 99 times. If the repeat value is less than or equal to 5 then results are sorted and some statistical values are calculated and printed after the sample results.

2.6.5 Number of replicates

To evaluate sample preparation errors, a number of replicates of a sample can be used. The results for each sample are output separately. After each group of replicate samples, some statistical values will be sent to output. The maximum number of replicates are 99.

2.6.6 Number of cycles

All the sample cassettes in the rack can be counted in repeating cycles. One cycle means counting of all cassettes in a batch once (i.e. those being counted with one protocol). A batch ends when the next cassette with a protocol ID is found. When the instrument has counted all the cassettes in this batch, it moves the rack up until the first cassette in the batch is found. Then it recounts the cassettes in the batch. Counting of the next batch starts when the cassettes have been counted the number of times specified by the Cycle parameter. The repeating cycles can be used to check sample stability. The maximum number of cycles are 99.

If the number of cycles is greater than 1, then you will be asked to give the Cycle delay. This is in minutes and is the time between when one cycle ends and the next one begins.

2.6.7 Order of operation of repeated counting

If you have set two or all three of the repeat, replicate and cycle parameters to values greater than 1 the order of operation is:

First, repeat counting of each sample. The output consists of the individual results for each repeat count for a sample followed by statistics for the repeat.

Second, statistics for all the replicates of the sample are calculated using the statistics for the repeat.

This procedure is repeated for all samples in an assay batch. When the end of the batch is reached, counting starts from the beginning by counting the repeats and replicates again. This procedure continues for as many times as you have defined for the cycle parameter.

2.6.8 Delay between plates

This parameter can be used to control the interval between when the counting of one sample plate has finished and the next is started. The range is 0 to 9999 minutes.

2.7 Crosstalk correction

2.7.1 Introduction

Crosstalk is a situation in which light pulses from adjacent samples interfere with the pulses of the sample under measurement. This may occur in the case of microtitration plates in which you cannot use the cassettes 1450-101 or 1450-102. This is because designs of cassette other than the 1450-101 or 1450-102 have part of the optical shielding between the wells removed to accommodate the microtitration plate.

Crosstalk can be corrected with the MicroBeta program. Before counting the actual samples, the amount and type of crosstalk is defined using special standardization or normalization samples, with the type of solution as similar to the actual samples as possible. The normalization or standardization is done first and the calculated crosstalk factors are then used when counting the actual samples. A CPM normalization run is needed for crosstalk corrected CPM results and a DPM standardization run is needed for crosstalk corrected DPM results.

2.7.2 Crosstalk CPM normalization

2.7.2.1 Preparation of normalization samples on a 96 or 384-well microtitration plate

Take sample solution with some activity (+ scintillant) which corresponds to the samples to be analyzed, i.e. has the same isotope and solvent, and pipette the same volume as in the samples, into well G11 (96-well plate) or well N22 (384-well, 1-6 detectors) or well M21 (384-well, 12 detectors).

For a 96-well plate with a 1-6 detector counter, pipette background solution (corresponding to the sample solution, but without activity) into wells A1, F11 and G10 (96-well), where the latter two are the crosstalk samples. G10 is only needed for strip plates and A1 if background correction has been selected. With a 12 detector counter use positions A1 and F11. Strip plate is not available. For a 384-well plate with a 1-6 detector counter use positions A1, M22 and N21 for strip plate. With a 12 detector counter use positions A1 and M22. Strip plate is not available.

The sample array for a 96-well plate with a 1-6 detector counter is as follows, where S1 is the sample solution and B1, C1 and C2 are the background solutions:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F											C1	
G										C2	S1	
H												

Close the sample plate with a sealing tape and place the plate on a 1450-103 or 1450-105 cassette for counting.

2.7.2.2 Preparation of normalization samples on a 24-well microtitration plate

The procedure is the same as with 96-well plates, but the normalization sample positions are D6 for the sample solution and A1, C6 and D5 for the background solution. D5 is only needed for strip plates; A1 is needed if background correction has been selected. The sample array is as follows:

	1	2	3	4	5	6
A						
B						
C						C1
D					C2	S1

2.7.2.3. Normalization Protocol

Edit a normalization protocol for crosstalk. To do this in the Ready-state press P (P)rotocols then N (N)ormalization protocols.

Select (E)dit and a protocol number.

Edit the protocol parameters, see the figure overleaf for an example of the parameters.

```
 1 Protocol name:           crosstalk correction
 3 Crosstalk correction (Y/N)                    Y
 5 Isotope 1:   1)H-3    2)I-125  3)C-14
                4)S-35   5)Cr-51  6)P-32
                7)P-32 Cerenkov   8)Other      1
                9)Luminescence
      PMT use:  1)Normal 2)Upper  3)Lower      1
10 Counting time for crosstalk [s]          60.0
11 Counting time for standards [s]          60.0
12 Precision (2 sigma) [%]                   0.2
30 Printer output:  1)No    2)Short
                    3)Long  4)Programmable     3
32 Display output:  1)No    2)Short
                    3)Long  4)Programmable     3
34 External output: 1)No    2)Short
                    3)Long  4)Programmable     1
36 File output:     1)No    2)Short
                    3)Long  4)Programmable     1
40 Change special features  (Y/N)              Y
41   Isotope activity setting  (Y/N)           N
42   Background sample  (Y/N)                  N
43   Half-life correction  (Y/N)               N
44   Chemiluminescence correction  (Y/N)       N
45   Use password  (Y/N)                       N
46   Special plate: 1)Wallac  2)BP filter
                    3)Other                    1
47   Strip plate  (Y/N)                        N
```

Select crosstalk correction on line 3, as well as the isotope (line 5) and counting times (lines 10 and 11). You can give the isotope activity setting on line 41 (Y for yes, and then the DPM value). A background sample is counted only if selected on line 42. If you use strip plates then select Y on line 47. The crosstalk sample in G10, D5 or N21 is counted only if strip plate is selected.

2.7.2.4 Normalization counting

Start the normalization run in the Ready or Count state using automatic counting and ID codes, or by selecting Nx, where x is the number of the protocol.

The normalization sample is then counted in every detector and the crosstalk sample in detector 1.

2.7.2.5 Counting the actual samples using crosstalk correction

Edit a counting protocol:

Select in the Ready-state P (P)rotocol then C (C)ounting protocols.

Then select E ((E)dit) and a protocol number.

On parameter line 2 select 1 (CPM) and on line 3 select the number of the crosstalk normalization protocol defined above. Edit the other parameters normally.

Start counting using automatic counting and a protocol ID label or start with the protocol number and press Enter.

When counting the samples, the stored crosstalk factors are used for correcting the CPM values. The crosstalk corrected CPM values are marked as CCPM1 in the printout.

2.7.3 Crosstalk DPM standardization

2.7.3.1 Preparation of DPM standardization samples on a 96 microtitration plate (1-6 detector counter)

Make first the standard samples in vials and then pipette them onto a plate:

a) 6 vials for isotope standard samples and 6 vials for blank (i.e. crosstalk) samples are needed.

b) Add into each standard sample vial (numbered from 1 to 6) scintillation liquid and isotope solution, or pipette the isotope solution directly into the sample wells, the same amount in each. Add the same solution into blank vials/wells, but without the isotope. (If there is no isotope in the standard vials, the same vials can be used for blank samples.)

c) Add quencher into each standard and blank vial, so that the samples form a quench series, i.e. the amount of quencher increases from vials 1 to 6. The amount of quencher added (here CCl_4 is used as a quencher) forms a series as follows:

Vial No.	Amount of CCl_4/5 ml µl
1	0
2	5
3	15
4	30
5	50
6	75

Shake the vials. Then pipette the standards and blanks onto a sample plate according to the following array (use the same total volume in standards and blanks as in the actual samples):

	1	2	3	4	5	6	7	8	9	10	11	12
A	C1		C2		C3		C4		C5		C6	
B	S1		S2		S3		S4		S5		S6	
C												
D												
E												
F												
G									S1			S6
H									C1			C6

The isotope standard samples are marked with S and a number, which refers to the vial number (the amount of quencher). The blank samples are marked with C and a number.

Close the sample plate with a sealing tape and place it on a 1450-103 counting cassette.

2.7.3.2 Preparation of DPM standardization samples on a 24-well microtitration plate

The procedure is similar to that described above but two plates are used and the sample positions are as follows:

First plate

	1	2	3	4	5	6
A	C1		C2		C3	
B	S1		S2		S3	
C						
D					S1	C1

Second plate

	1	2	3	4	5	6
A	C4		C5		C6	
B	S4		S5		S6	
C						
D					S6	C6

2.7.3.3 DPM standardization protocol

Make a DPM standardization protocol by selecting in the Ready-state P (P)rotocols then S (S)tandardization protocols.

Select edit (E) and a protocol number.

Edit the protocol, see the figure below.

```
Line (1..40, type / to exit, ? for help)      1 ->
 1 Protocol name:
   ->
 2 Sample type: 1)Normal 2)SPA                1 ->
 3 Crosstalk correction (Y/N)                 N ->Y Select crosstalk
correction
 5 Isotope 1:  1)H-3    2)I-125  3)C-14
               4)S-35   5)Cr-51  6)P-32
               7)P-32 Cerenkov   8)Other      1 ->
     PMT use:  1)Normal 2)Upper  3)Lower      1 ->
     Window 1:                          5- 320
10 Counting time for crosstalk [s]          60.0 ->
11 Counting time for standards [s]          60.0 ->
12 Precision (2 sigma) [%]                   0.2 ->
20 Number of standards                         6 ->
21 Isotope 1 activity [DPM]           200000.0 ->  Activity of standards is
                                                   given on line 21
24 Standard curve fit selection  (Y/N)       N ->Y Curve fitting method
                                                   given here
25   Curve fit method: 1)Smoothing spline
                       2)Interpolation spline
                       3)Linear interpolation
                       4)Linear regression    1 ->
     Automatic smoothing  (Y/N)              Y ->
26   Edit standard curve  (Y/N)              N ->
30 Printer output:  1)No    2)Short
                    3)Long  4)Programmable    3 ->
32 Display output:  1)No    2)Short
                    3)Long  4)Programmable    3 ->
34 External output: 1)No    2)Short
                    3)Long  4)Programmable    1 ->
36 File output:     1)No    2)Short
                    3)Long  4)Programmable    1 ->
40 Change special features  (Y/N)             N ->
```

Select crosstalk correction on line 3. Remember also to edit the parameters: isotope, counting time, number of standards and the isotope activity. See the listing above.

2.7.3.4 DPM Standardization Counting

Start the DPM standardization run in the Ready-state or Count-state using automatic counting and ID-labels, or by selecting Dx, where x is the protocol number (no ID-label is needed in this case).

The active samples are automatically counted first, e.g. positions G9, B1-B11 and G12, then the crosstalk samples A1-A11, H9 and H12.

2.7.3.5. Counting the actual samples with crosstalk correction

In the Ready-state select a counting protocol to edit.

On parameter line 2 select DPM-mode and on line 3 the crosstalk DPM standardization protocol defined above.

Start automatic counting using ID-labels or start with the protocol number and press Enter.
The DPM values in the printout are the crosstalk corrected values.

2.8 Datafiles

2.8.1 Use

Counting results can be stored on a floppy or hard disk located in the PC (terminal emulation or MultiCalc); they can also be sent to the server of a local area network (LAN). Results can also be stored on the MicroBeta disks in drive A: or B:. If a VT52 or VT100 terminal is used then results are stored on the protocol disk in MicroBeta drive B:. (see 2.31.6.4 Terminal). The data in the files saved on the disk is available to be processed or printed.

Data can also be sent to an external computer. The data is sent via port 2 on the back of the counter.

Note: Port 1 is for the Terminal PC. The third output, port 3, is for the printer.

2.8.2 Disk drive selection

The disk drive of the terminal PC where the data is to go can be chosen in the System state by pressing D (Data drive). You can choose from drives A, B or C where C is a hard disk drive and A and B are for floppy disks. You can also specify the whole directory path (see the chapter System/(D)ata drive). Start the path with @ if you wish to store results on the MicroBeta disk.

Depending on the type of PC you have to proceed as follows:

Single floppy disk PC - You must select drive A. Then you must replace the terminal emulator program diskette with an empty but formatted data diskette.

Dual floppy disk PC - Select drive B. This allows you to keep your terminal emulator diskette in drive A. Load an empty formatted data diskette into drive B.

Hard disk PC - Select drive C (hard disk) or specify the path. This will allow you to collect large amounts of data. If you have only specified the drive you must select the directory on the terminal PC where you want to store your data. To do this, exit from the terminal emulator program. (See the chapter Terminal emulators/Exit to MS-DOS). Use the change directory command in MS-DOS i.e. cd\directory name to go to the directory you want the data to be stored in. When you have done this, then return to the terminal emulator program by typing EXIT and pressing Enter.

The disk drive and the directory for the results files can also be selected in the protocol together with the output file selection. This will override the selection made in the System state and will make it possible for users to have their own results files directories

When you have selected the directory you can instruct the program to save data on it as follows.

2.8.3 Saving data in datafiles.

Select protocol operations by typing P, N or S when in the Protocols state. (P is for counting protocol, N for normalization protocol and S for DPM standardization protocol).

Next choose the protocol to be edited by giving the group number and pressing Enter.

You can edit any parameters in the protocol but in particular go to line 36 File output and select which data you want to save (see chapter 2.22 Protocols for details). The example shows that the Long output has been selected. Then on the line File path enter the disk drive and directory or leave it as it is if the drive defined in the System/Data drive shell is used. In this example a subdirectory TEST belonging to directory MB on PC disk A has been selected.

```
36 File output:     1)No    2)Short
                    3)Long  4)Programmable    1 ->3
     File path:              C:\MB\->A:\MB\TEST\
     File name extension                      1 ->
40 Change special features  (Y/N)            N ->
```

Before starting counting be sure that you have inserted an empty formatted data diskette into the drive selected or, if you have selected saving to hard disk (drive C:), be sure that the current directory has been selected as described in the previous section. If the file path is given, then this directory does not need to be the current directory, but it must exist. Use the MKDIR command in MS-DOS to make new directories. Format new diskettes by the MS-DOS FORMAT command.

When you have finished protocol editing, store the protocol by pressing / and begin counting by pressing A in the Ready or Count state.

Note: Line 36 is not available if UltroTerm is used. The filing system of UltroTerm should be used in this case.

2.8.4 Accessing datafiles

Result files that are stored on the data disk will be named so that the first part of each name (the part before the point) is the protocol number and the second part (after the point) is a

running number indicating the assay execution order. E.g. for protocol 5 the first saved file is named 5.001, the second 5.002 etc. Other files have the format e.g. S5.001 for standardization files and N5.001 for normalization files. The file name extension can be changed in the protocol. To access a file on the data disk in the terminal PC, exit from the terminal emulator program to MS-DOS (see the chapter Terminal emulators/Exit to MS-DOS).

2.8.5 External datafiles

Results can be sent to an external data collection device by connecting it to port 2. The external output and the result format are chosen on protocol line 34 External output.

```
34 External output: 1)No    2)Short
                    3)Long  4)Programmable    1 ->4
35 External output: POS  CCPM1  CCPM1%
->POS  CTIME  CPM1  CPM1%  CCPM!  CCPM1%
```

There are four alternatives which you can select on protocol line 34 External Output:

1. Nothing is sent

2. Only position number, CCPMs and CCPM%, DPM and DPM%, or LCPS, LCPS% and FLAG are sent (CCPM is corrected CPM and % means the percentage error in the CCPM, DPM or LCPS value). CCPM, DPM or LCPS are also sent in PLATE format

3. The long results printout including SQP(I) and CPMs is sent (see the Results chapter)

4. User selected outputs are sent

2.8.6 Data processing

Counting results are stored in ASCII format, and they can be processed using commercial spreadsheet programs.

2.8.7 Datafiles in MultiCalc

Results from MultiCalc controlled Assay protocols are handled in a different way. The types of results files are selected in the Assay protocol editor. Input is an internal file for saving data to be used later in MultiCalc, e.g. in evaluations. Results is a text file to be used mainly for transferring data to an external computer. See the User Guide to MultiCalc functions manual and the module called File handling and evaluation.

Results from MicroBeta controlled protocols are handled as with any other terminal emulator, i.e. they are sent via MultiCalc to the drives and directories specified in the protocols. These result files cannot be evaluated by MultiCalc.

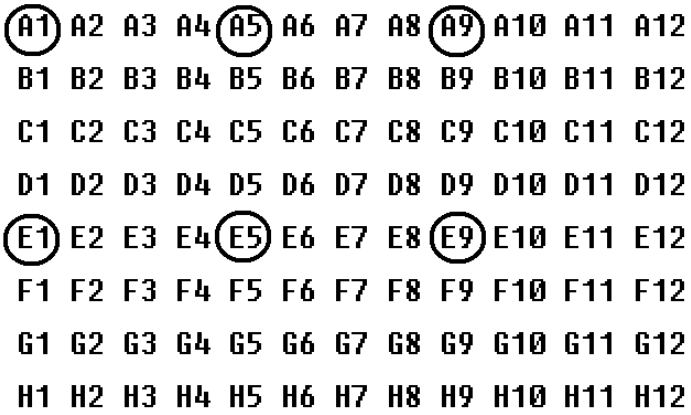
2.9 Detectors

2.9.1 Six detector model

This model of MicroBeta has twelve photomultiplier tubes arranged in six pairs. Each pair works in coincidence. The tubes of the pair are situated on opposite sides of the counting block, one above and one below the block. The detectors form a 3 x 2 array, see the figure below.

Six samples are counted simultaneously, which reduces counting time to about one sixth of that for a single detector instrument.

When a cassette is being counted, the transportation system moves it between the detector blocks. The first detector then counts position A1, the second A5 (A3 for 24-well plates), the third A9 (A5), the fourth E1 (C1), etc. (see the figure below). At this phase only the results from position A1 are output, because the results must be in the order A1, A2, A3, etc. The cassette moves one position forward and positions A2, A6, A10, E2, E6, and E10 are counted. The results from A2 are output. After counting the whole row the cassette moves to the beginning of the next row (e.g. position B1 in detector 1). The rest of the results from row A are output.



Six detector model, cassette in first position

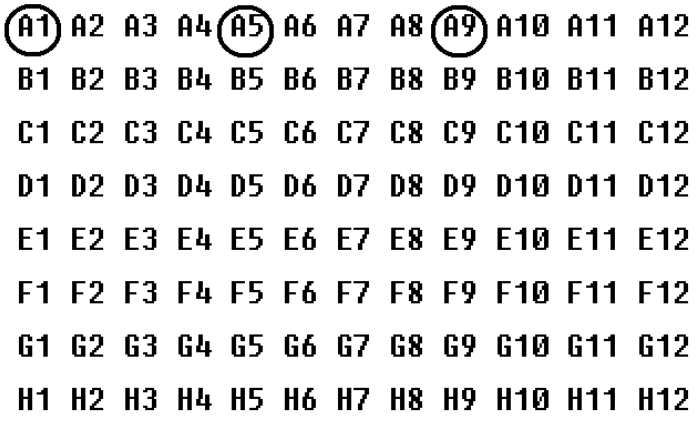
Only the first detector can count every position in a plate. The following table shows which positions can be counted by each detector:

Det.No.	96-well	24-well	384-well
1	every	every	every
2	A5-A12 ..... H5-H12	A3-A6 ..... D3-D6	A9-A24 ..... P9-P24
3	A9-A12 ..... H9-H12	A5-A6 ..... D5-D6	A17-A24 ..... P17-P24
4	E1-E12 ..... H1-H12	C1-C6 D1-D6	I1-I24 ..... P1-P24
5	E5-E12 ..... H5-H12	C3-C6 D3-D6	I9-I24 ..... P9-P24
6	E9-E12 ..... H9-H12	C5-C6 D5-D6	I17-I24 ..... P17-P24

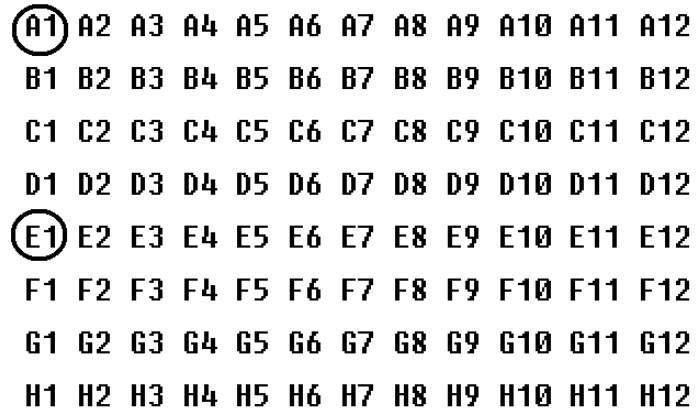
The smallest range of positions is that counted by detector 6. This is therefore the only range that can be counted by all detectors. This affects the positioning of CPM normalization and DPM standardization samples because these routines involve every detector counting the same sample. See chapters 2.12 and 2.23 for details.

2.9.2 Three, two and one detector models

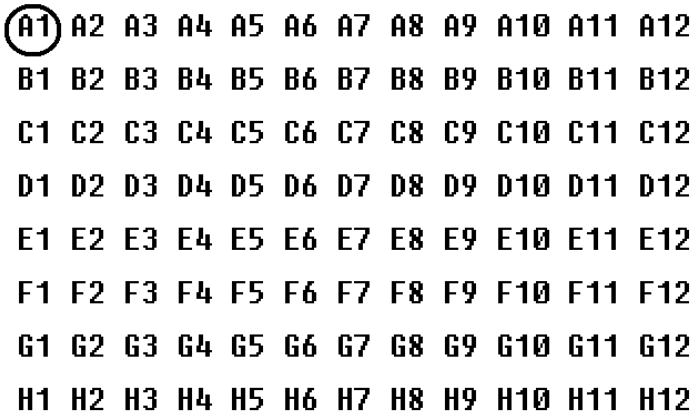
The MicroBeta series also includes models with 3, 2 and 1 detectors. The detectors are then arranged as shown in the following figures.



Three detector model, cassette in first position



Two detector model, cassette in first position



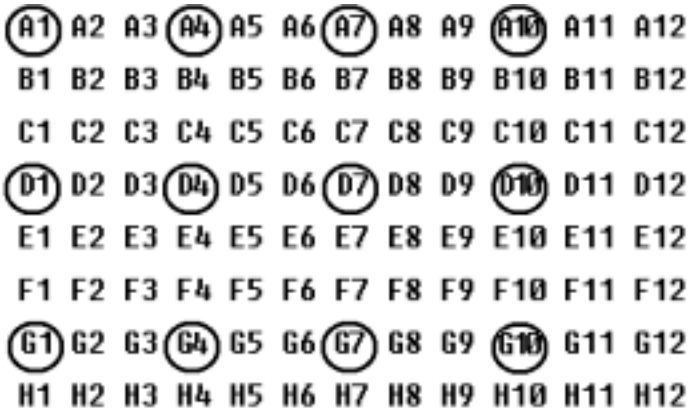
One detector model, cassette in first position

2.9.3 Selecting detector number

The amount of detectors must be defined in the system parameters before starting counting. Go to the System state by pressing S and select the test level by pressing L followed by T. Then select detector usage by pressing U and choose one of the alternatives for the number of detectors. Return to the normal level by pressing L followed by N before quitting to the Ready state by pressing Q.

2.9.4 Twelve detector model

A twelve detector MicroBeta has twenty-four photomultiplier tubes arranged in twelve pairs. Each pair works in coincidence. The tubes of the pair are situated on opposite sides of the counting block, one above and one below the block. The detectors form a 4 x 3 array, see the figure below.



Twelve detector model, cassette in first position

Twelve samples are counted simultaneously, which reduces counting time to about one twelfth of that for a single detector instrument. There are nine readings per plate.

When a cassette is being counted, the transportation system moves it between the detector blocks. The first detector then counts position A1, the second A4 etc. At this phase only the results from position A1 are output, because the results must be in the order A1, A2, A3, etc. The cassette moves one position forward and positions A2, A5 etc. are counted. The results from A2 are output. After counting the whole row the cassette moves to the beginning of the next row (e.g. position B1 in detector 1). The rest of the results from row A are output.

A limited range of positions (10 to 12 in row G) can be counted by all detectors. This affects the positioning of CPM normalization and DPM standardization samples because these routines involve every detector counting the same sample. See chapters 2.12 and 2.23 for details.

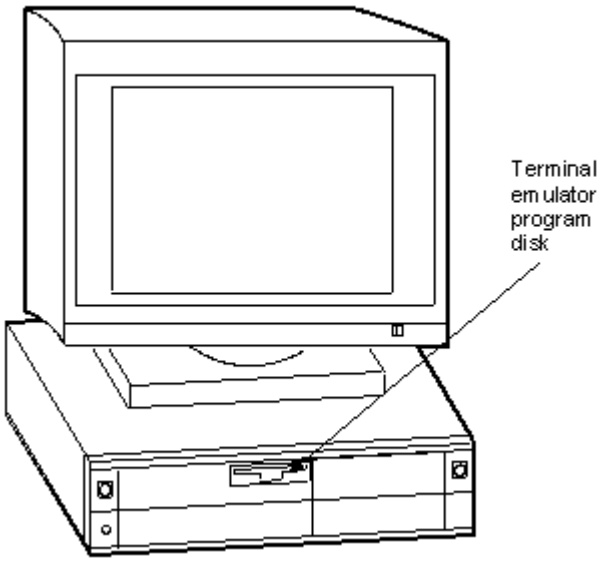
2.10 Diskettes

2.10.1 Diskettes used

The diskettes used in the MicroBeta counter itself are 3.5" micro-floppy disks with standard 1.44 MB format. The program diskette has to be in drive A (the lower drive) and the protocol diskette in drive B (the upper drive).

A diskette is inserted into a disk drive with the label upwards and towards you. When the diskette is properly in its place, a click is heard. To remove the diskette press the button under the diskette slot.

Terminal emulator diskettes are normally 3.5" (1.44 MB) micro-floppy disks and these are inserted into the A drive of the terminal PC.



2.10.2 Diskette handling and storing

A floppy disk should be handled carefully. Avoid touching the magnetic surface, and always when handling the diskette touch only the end with the label on. Do not bend the diskette, or write on it. When labelling it the label should be written first and affixed afterwards on the diskette. Do not place anything heavy on it (e.g. books). Put the diskette in a cardboard envelope when not in use. Diskettes should be stored in a closed box to avoid dust getting on them. The storage temperature should be between 10 - 30^{o} C. They should also be kept away from magnets.

2.10.3 Backup of the terminal diskette

To make a backup copy of the terminal program or data diskette in the terminal PC use the MS-DOS DISKCOPY command (see 2.31.2.8 Terminal emulators/ Backup).

2.10.4 Backup of the program diskette

There should always be a backup copy of the program diskette in case something should happen to the original. To make a backup copy, start the MicroBeta program as normal. Then type S to get to the System state and there type O for exit to MS-DOS. Press Y twice to confirm the exit. After the prompt MicroBetaA:\> appears on the screen type PROGRAM, and press Enter. Follow the instructions on the screen. Put a new diskette into drive B: so that a copy can be made. Store the new copy of the program diskette in a safe place.

To get back to the MicroBeta program put the protocol diskette back into drive B, type MB and press Enter.

2.10.5 Backup of the protocol diskette

Protocol modifications are saved on the protocol diskettes so it is advisable to make a backup copy of these diskettes from time to time in order to save the protocols used.

To make a backup copy of the protocol diskette, begin in the same way as with the program diskette. The procedure differs from the point when the prompt MicroBeta A:\> is displayed. Here type PROTOCOL BACKUP and press the Enter key. Remove the program diskette and put a new protocol diskette into drive A to receive the protocols.

If you want to make a new empty protocol diskette, then type PROTOCOL NEW instead, and press the Enter key. Then follow the instructions displayed on the screen.

Now store the copied diskette as a backup copy, and use the other diskette as a protocol diskette in MicroBeta, or, if a new empty protocol diskette was made, use it for saving new protocols.

To get back to the MicroBeta program, put the program diskette back into drive A, type MB and press Enter.

2.10.6 Preparing a 3.5 protocol diskette

A standard 3.5" MS-DOS 1.44 MB formatted diskette can be used as a MicroBeta protocol diskette. When you want to initialize an empty formatted diskette to be used as a protocol diskette, insert the empty diskette into drive B on the counter and start up MicroBeta. When the program has been loaded, the following message should appear:

```
Cannot find the protocol index
```

Press M. The program will try to find the protocols on the diskette and will create the protocol index. Then the program returns to the Ready state and the protocol diskette is ready for use.

2.11 DPM counting

2.11.1 Why DPM counting?

CPM counting is used when sample preparation is expected to yield samples with close to constant counting efficiency, i.e. no variation in quench level. This means that the results of the samples in an assay can be compared with each other and used in further data analysis. If the counting efficiency varies with quench in the sample, CPM counting can no longer give accurate results, DPM counting is therefore needed.

DPM counting involves the use of quench curves. These are made after counting a number of standards with different quench levels. This procedure is called DPM standardization. The spectra from counting unknown samples are compared with the quench curve and accurate results are calculated.

In MicroBeta the quench curves are constructed by first counting 2 to 12 DPM standards in detector 1 and then counting two so called DPM normalization standards in every detector. The curve from detector 1 is recalculated for the other detectors depending on the detector efficiencies. See chapter 2.12 DPM standardization for more information about the standardization procedure.

2.11.2 Single label DPM

The DPM standards are counted in detector 1. MicroBeta has no external standard so the spectrum quench parameter for the isotope (SQP(I)), or the Asymmetric quench parameter for the isotope, AQP(I), is therefore used for determining the quench curve. AQP(I) is used when ParaLux counting is selected. The normalization standards are then counted in every detector in order to get the efficiencies and SQP(I) (or AQP(I)) values.

Later, when measuring an unknown sample, the CPM and SQP(I) (or AQP(I)) values are counted in a particular detector. SQP(I) (or AQP(I)) is modified to fit as SQP(I) (or AQP(I)) for detector 1. The efficiency for detector 1 is counted from this SQP(I) (or AQP(I)) by using the quench curve, and is then used for determining the efficiency for the particular detector. The DPM value is counted by dividing CPM with the efficiency. See chapter 3.3 Calculation methods for formulae used in DPM counting.

2.11.3 Easy DPM

Easy DPM is a special case of single label DPM counting. Only normalization standards are counted in standardization and stored quench curves are used when counting unknown samples.

2.11.4 Dual label DPM

Dual label counting is similar to single label counting except that two plates are used, one for each isotope. The DPM1 and DPM2 values are calculated using the 3/2 method.

Note: EasyDPM is not available with ParaLux count mode.

2.12 DPM Standardization

2.12.1 What is standardization?

When counting DPM samples in a multidetector counter the variation in detector efficiency, detector energy and the effect of quenching must both be corrected for. Standardization is the name given to the process in MicroBeta which makes this correction.

2.12.1.1 Quench correction

Several samples labelled with the same activity will give different count results even in the same detector because of the difference in the amount of energy absorbed in the samples. This is called quenching. To correct for this a number of samples with identical DPM but differing quench have to be counted in one detector. This establishes a relationship between a quantity called SQP(I) or AQP(I) (the latter is used for ParaLux counting) and efficiency. SQP(I) (or AQP(I)) is a measure of the isotope spectrum and varies as the quench changes. Once this relationship between SQP(I) (or AQP(I)) and efficiency is known it is possible to calculate the counting efficiency for any measured SQP(I) (or AQP(I)) value in the range covered by the measurement of the standard samples. Knowing the efficiency, the DPM can be calculated.

2.12.1.2 Detector energy and efficiency calibration

However closely matched detectors are, there will be some difference in their energy calibration (affecting SQP(I) (or AQP(I)) measurement) and counting efficiency. A correction must be made for both these differences.

2.12.1.3 Standardization

Standardization = Detector energy calibration + Sample quench correction + detector efficiency calibration

In MicroBeta the standardization is done by first measuring a maximum of 12 DPM standards with defined activities in detector 1 and then counting two DPM standards in every detector. The two calibration standards should be representative of the range covered by the DPM standards, i.e. they could be replicates of the first and last DPM standards.

The quench standard measurement produces a curve of efficiency versus SQP(I) (or AQP(I)) with as many points as standardization samples.

Energy calibration and efficiency calibration are both linear functions so two points are sufficient to define these two calibration curves. The two points are obtained from the counting of the two calibration standards mentioned earlier.

It is preferable that each standard is counted with the same amount of pulses to get an accurate curve. Set the counting precision on line 12 of the counting protocol. Make sure the counting time is long enough that the precision requirement will terminate counting and not the counting time. See chapter 2.25 Protocols section 2.25.7 number 12 for more details.

2.12.1.4 Easy DPM

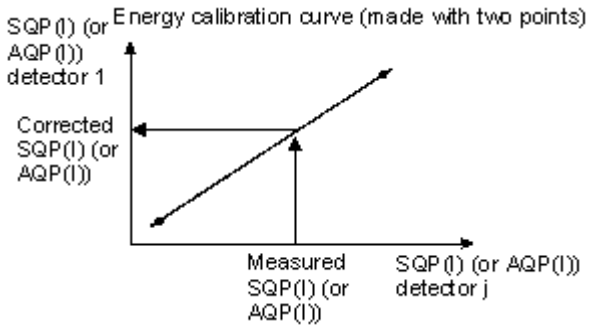
If Easy DPM is selected, then DPM standards are not needed. The instrument uses stored quench curves for isotopes ^{3}H, ^{125}I, ^{14}C, ^{35}S, and ^{32}P. Only two normalization samples are measured in standardization. Normalization sample positions are H11 and H12 (96-well plate 1-6 detectors), G11 and G12 (96-well plate, 12 detectors), D5 and D6 (24-well plate), P23 and P24 (384-well plate, 1-6 detectors) M22 and M23 (384-well plate, 12 detectors).

2.12.1.5 Evaluating unknown samples

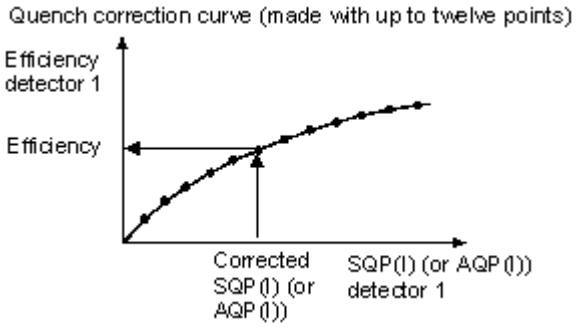
The actual sequence of events for counting unknown DPM samples is as follows:

1 The SQP(I) (or AQP(I)) value and CPM are obtained with any detector.

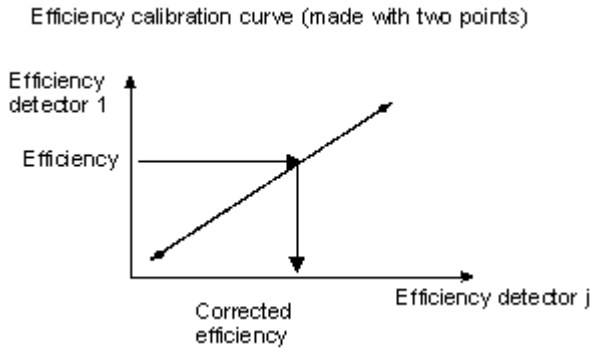
2 The SQP(I) (or AQP(I)) value is now corrected from the energy calibration curve to be what it would have been if the sample had been counted in detector 1.


Energy calibration curve (made with two points)
SQP(I) (or AQP(I)) detector 1
Corrected SQP(I) (or AQP(I))
Measured SQP(I) (or AQP(I))
SQP(I) (or AQP(I)) detector j

3 The corrected SQP(I) (or AQP(I)) is used to read off the detector 1 efficiency from the quench curve made for samples counted with detector 1, see the figure overleaf.


Quench correction curve (made with up to twelve points)
Efficiency detector 1
Efficiency
Corrected SQP(I) (or AQP(I))
SQP(I) (or AQP(I)) detector 1

4 The efficiency calibration curve is then used to obtain the actual detector efficiency corresponding to the detector 1 efficiency just obtained.



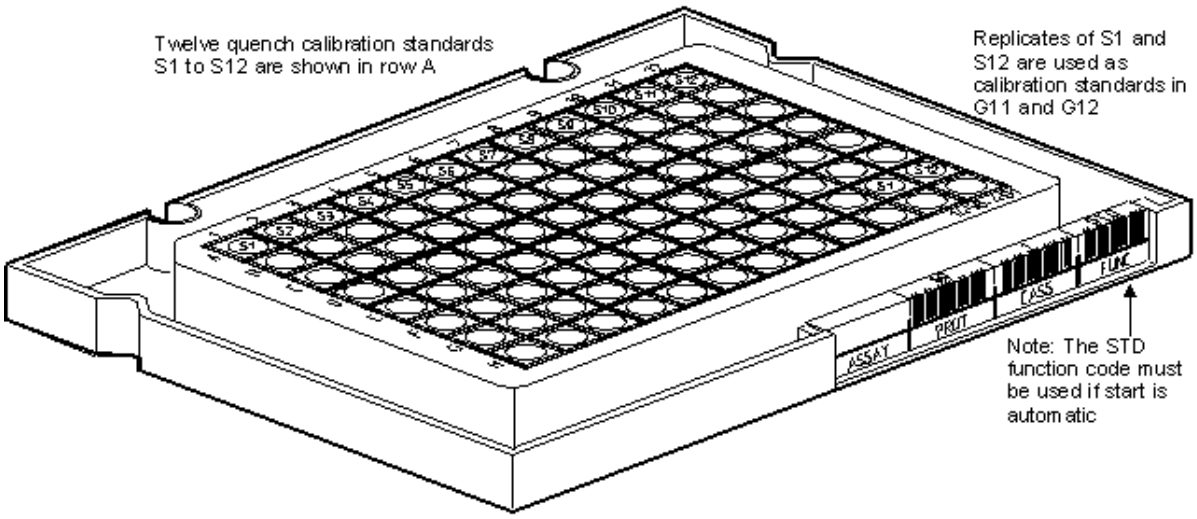
5 The measured CPM value is then divided by the actual detector efficiency to obtain the activity in DPM.

2.12.2 Editing standardization parameters

Standardization parameters are stored in standardization protocols (see the listing in section 2.12.3). There can be a maximum of 100 standardization protocols, numbered from 0 to 99. The default protocol is number 0.

Protocols 91 to 95 , 97 to 98 are made at the factory for isotopes ^{3}H, ^{125}I, ^{14}C, ^{35}S, ^{32}P and ^{32}P Cerenkov respectively. These may not be deleted because they are connected to the corresponding counting protocols. Note that no standardization has been done for those protocols i.e. there is no standardization data connected to the protocols. Standardizations must actually be performed with the protocols to get the standardization data.

Press P and S in the Ready state to choose the standardization protocol mode. The protocols can then be edited, copied, deleted, printed and displayed, and the curves can be plotted.



Positions of standardization samples in a 12-detector MicroBeta

Press the appropriate key for the following options:
F - default parameters
E - edit a protocol
C - copying a protocol including standardization data
D - deleting a protocol

Note: the standardization data will be destroyed when editing an old standardization protocol. The standardization must therefore be done again for this protocol. Only the standardization protocol name, curve fitting method, the curve and password can be edited without destroying the standardization data.

See chapter 2.25 Protocols for further details on standardization parameters and editing.

2.12.3 Preparing single label standardization samples

Two to 12 DPM standards are placed in positions A1 to A12 in a 96 or 384-well plate or in positions A1 to B6 in a 24-well plate. No empty positions in the beginning or between samples are allowed. The first and last normalization standards must be also in position H11 and H12 (96-well, 1-6 detectors), G11 and G12 (96-well, 12 detectors), D5 and D6 (24-well), P23 and P24 (384-well, 1-6 det.) and M22 and M23 (384-well, 12 det.). If Easy DPM is selected then only normalization standards are needed.

If ParaLux counting is selected, then it is possible to subtract thermal background from the AQP(I) and counts values. Select Background sample on line 42 and put an empty cassette as the first standardization cassette followed by the usual standardization cassette.

```
Line (1..40, type / to exit, ? for help)      1 ->
 1 Protocol name:
   ->
 2 Sample type: 1)Normal 2)SPA                1 ->
 3 Crosstalk correction (Y/N)                 N ->
 4 Number of labels:  1)Single  2)Dual        1 ->
 5 Isotope 1:  1)H-3    2)I-125  3)C-14
               4)S-35   5)Cr-51  6)P-32
               7)P-32 Cerenkov   8)Other
               9)Luminescence                 1 ->
     ParaLux  (Y/N)                           Y ->
       ParaLux mode:  1) High efficiency
                      2) Low background       2 ->
       Discriminator channel                150 ->
     PMT use:  1)Normal 2)Upper  3)Lower      1 ->
     Window 1:                          5- 360
10 Counting time for crosstalk [s]          60.0 ->
12 Precision (2 sigma) [%]                   0.2 ->
14 Easy DPM  (Y/N)                             N ->
20 Number of standards      •                  6 ->
21 Isotope 1 activity [DPM]            200000.0 ->  Activity of standards is
                                                    given on line 21
24 Standard curve fit selection  (Y/N)         N ->Y Curve fitting method
                                                    given here
25   Curve fit method: 1)Smoothing spline
                       2)Interpolation spline
                       3)Linear interpolation
                       4)Linear regression     1 ->
     Automatic smoothing  (Y/N)                Y ->
26   Edit standard curve  (Y/N)                N ->
30 Printer output:  1)No    2)Short
                    3)Long  4)Programmable     3 ->
32 Display output:  1)No    2)Short
                    3)Long  4)Programmable     3 ->
34 External output: 1)No    2)Short
                    3)Long  4)Programmable     1 ->
36 File output:     1)No    2)Short
                    3)Long  4)Programmable     1 ->
40 Change special features  (Y/N)              N ->Y
41   Isotope activity setting  (Y/N)           N ->
42 Background correction (Y/N)                 N ->Y
     Use normalization background (Y/N)        N ->
     Background sample (Y/N)                   N ->Y   Background sample
     Bgnd sample position                     G12 ->    selected
     Bgnd counting time [s]                    60 ->
43   Half-life correction  (Y/N)               N ->
44   Chemiluminescence correction  (Y/N)       N ->
45   Use password  (Y/N)                       N ->
46   Special plate: 1)Wallac  2)BP filter
                    3)Other                    1 ->
47   Strip plate  (Y/N)                        N ->
```

2.12.4 Preparing dual label standardization samples

Two plates are used and the higher energy isotope samples are counted first. A maximum of 6 DPM standards can be used on each plate. For 24-well plates the sample positions are the same as in single label standardization.

For a 96-well plate the standards are placed in the following positions on each plate: B1, B3, .. , B11, the first normalization standard in position G9 (96-well, 1-6 det.), G10 (96-well, 12 det.) and the last normalization standard in position G12 (96-well 1-12 det.).

2.12.5 DPM standardization procedure

The plate(s) with the standardization samples are placed in cassette(s) which are identified with the corresponding standardization protocol number in the protocol number area and with a STD label in the function code area. In dual label standardization the first (higher energy isotope) cassette should have the STD label or STD2 for the first plate and STD1 for the second. Insert the cassette(s) into the rack and close the door. Press either A (Automatic counting) or Dxx, where xx is the standardization protocol number, in the Ready or Count state to start counting. If Dxx is selected the program asks:

```
Do you want to continue counting after standardization? (Y/N)->
```

If Y (Yes) is selected the counting continues after counting the standardization plate, if N (No) is selected the counting stops after the standardization.

If standardization has already been done for the protocol, then the program asks first:

```
This protocol contains standardization data. Continue? (Y/N) ->
```

This is to prevent accidental overwriting of standardization data.

If automatic counting is selected the standardization cassette can be placed anywhere in the rack. The STD label is needed in this case.

The counting results for the standardization are output as specified on lines 30 - 37 in the standardization protocol.

2.12.5 Standardization results

When a standardization is done the results are stored with the standardization protocol. The standardization data can be used by one or several counting protocols and is selected when editing the protocol (see chapter 2.25 Protocols).

2.13 Errors

2.13.1 Program failure

If the program is jammed and will not take any orders typed on the keyboard, it is best to restart the program. Turn the power off from the counter and the terminal. Switch the power on again, first the terminal and then the counter and try again. If the counter does not restart then remove the battery fuse before switching the power on.

2.13.2 Pressing the wrong keys

If a wrong key is pressed, the beep is heard and alternatives are shown again. If in protocol editing a wrong value is given, it can be easily erased by pressing the Backspace key and retyping the correct value. If the Enter key has already been pressed, then type L and the line number to return to the line to enter a new value or either the up arrow or P and Enter to go to the previous line.

2.14 Half-life

2.14.1 Radioactive half-life

The half-life of a radioactive isotope is the time in which half of the radioactive nuclei have decayed, i.e. the activity is halved. The decrease in activity is exponential and can be calculated using the formula:

$$A(1) = A(0)x2^{(-t/T_{half})}$$

Where: A(1) = the activity at time t(1), A(0) = the activity at time t(0) and t=t(1) - t(0). T_{half} = the half-life of the isotope.

Each radioactive isotope has its specific half-life. If the half-life of an isotope to be counted is short, and if the counting time of a batch of samples is relatively long, the last samples will have decayed more than the first ones so the results between earlier and later samples cannot be directly compared with each other. As an example: the half-life of the ^{32}P isotope is 14.3 days, and if counting takes 24 hours, the last samples will give about 5 % lower activity than they would have done if counted at the same time as the first sample.

2.14.2 Half-life correction

The half-life correction is calculated, if requested, during protocol setting. This correction is recommended if the half-life of the isotope used is short and/or the total counting time is long.

If you select one of the six defined isotopes in protocol setting, the half-life will appear on line 43, see the figure.

```
40 Change special features  (Y/N)              N ->Y
41   Isotope activity setting  (Y/N)           N ->
42   Background sample  (Y/N)                  N ->
43   Half-life correction  (Y/N)               N ->Y
       Half-life 1 (h)                107500.00 ->
         Zero time 1 set  (Y/N)               N ->
           Zero date 1                28-Jun-2000 ->
           Zero time 1                   15:40:53 ->18:30
44   Chemiluminescence correction  (Y/N)       N ->
45   Use password  (Y/N)                       N ->
46   Special plate: 1)Wallac  2)BP filter
                    3)Other                    1 ->
```

Tritium was the isotope selected on line 5 and the half-life is given automatically after line 43

If you select isotope option 7 "Other" then you must specify the half-life in hours on line 43. If you type "0.0" no half-life correction will occur. The next line asks if you want to set a zero time. If you select Y then two more protocol lines appear to allow you to set the zero date and

zero time. This corresponds to time t(0) described earlier in the formula. This is the moment back to which all results are corrected. If you select N to the zero time setting question then activities are corrected to the time when counting of the batch of samples was started.

Isotope	Half-life	Half-life hours
^{3}H	12.3 years	107500
^{14}C	5760 years	-
^{32}P	14.3 days	343
^{35}S	87.2 days	2093
^{51}Cr	27.8 days	667
^{125}I	60.0 days	1440

If you select a suitable half-life correction then, for example, if a batch is recounted after e.g. one week, the corrected CPM results are directly comparable with each other since they are half-life corrected back to the same date and time.

N.B. Always check that the date and time are set to correct values in MicroBeta. The current time can be seen in the upper right corner of the screen during counting. If these values are wrong, set them in the System state (see Clock).

2.15 Help and Info

2.15.1 MicroBeta TriLux Help

The MicroBeta program has a Help function. This means that information can be displayed to explain what options are open to you at the phase of operation you are in. This information can be obtained by pressing one of the keys: H, h or ?. In protocol editing press Enter also. These help keys can be pressed at any stage of the program with one exception. The H keys must not be used to get help when H has another meaning e.g. when defining active rows in the protocol editor. Use ? to get help at that point.

2.15.2 MultiCalc Help

At any point in using MultiCalc you can get help by pressing the F10 function key. To get help about the function of any softkey press the ALT key and the softkey at the same time. Pressing ALT and F9 gives you a general keyboard help at any part in the program when text mode is being used for the screen.

To clear a help press ALT and F10.

As experience in operating the instrument grows with time, the need for detailed information decreases, and the additional information the Help function gives will not need to be accessed so often.

2.15.3 Selecting info

Info contains a short user manual and information on consumables, normalization and standardization positions, changes after the previous program version and customizing the program.

Information can be viewed on the screen or printed. To enter the Info state press I in the Ready state.

```
Ready>I

(H)elp     (Q)uit
(U)sage    (C)onsumables   (P)ositions
Ch(a)nges  Cu(s)tomizing   S(y)stem

Info>
```

2.15.4 (H)elp

Press H to get help about the various selections.

2.15.5 (U)sage

This is a short user's manual with basics on how to use the instrument.

2.15.6 (C)onsumables

This is an up-to-date list of the currently available consumables.

2.15.7 (P)ositions

This is a list of sample positions in standardization and normalization.

2.15.8 Ch(a)nges

This is a list of additions and changes made to the program after the previous version.

2.15.9 Cu(s)tomizing

The program can be customized using various environment strings. These strings and their effects are listed under this control letter.

2.15.10 S(y)stem

This is a list of system information and settings.

2.16 Interrupt

2.16.1 Stop

If it is necessary to stop counting quickly, press O (Off). The cassette currently in the counting position will be moved back to the rack and the program will enter the Ready state.

Note: If you have started counting from MultiCalc and are in MultiCalc mode, you cannot use the O key. Instead you must press F1 (=STOP), or if the softkeys are not in use, then press any other key e.g. the space bar to stop counting.

2.16.2 Open the door

If the door is opened during counting, the high voltage is cut off, and the counting stopped. An error message is displayed on the screen. When the door is closed the program starts counting the current positions from the beginning.

2.16.3 Next position or assay

If you want to stop the counting of the current samples, press key N (Next position) or E (Next assay). Counting will be interrupted and the MicroBeta will start to count the next position if N is pressed or the next assay if E is pressed. If quick view is used and N(Next plate) is pressed, then MicroBeta starts counting the next plate. Similar keys in MultiCalc mode are F2 (= NEXT POS) and F3 (= NEXT ASSAY).

2.16.4 Causing a pause in printing

It is possible to cause a pause in printing during counting. Press P (Pause printing). After a while the computer asks if you want to pause in printing. Answer Y (Yes) to the question to cause a pause in printing. To resume printing later on press P again. To not pause, answer N (No). This feature is not included in MultiCalc mode.

2.16.5 Causing a pause in data transfer

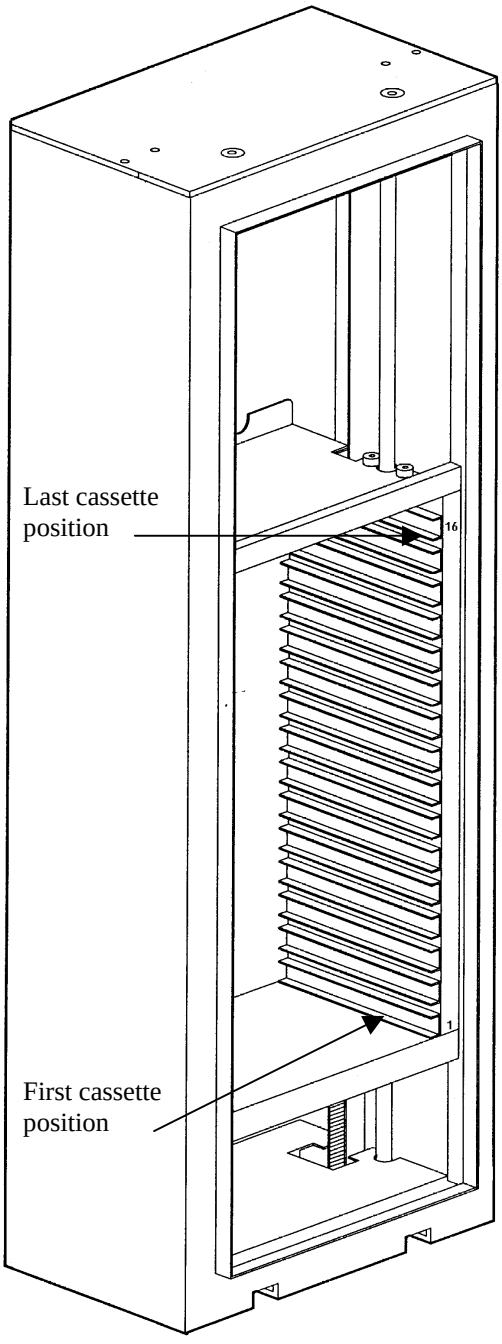
A pause in data transfer to the terminal PC can be caused if required. It is recommended that this feature be used before making a temporary exit from the terminal emulator program to the MS-DOS of the terminal PC. MicroBeta can measure approximately three cassettes belonging to the same assay after the pause in data transfer has occurred. If the pause is longer than this, then more results will not be saved.

To cause a pause in data transfer press D (Disconnect) and answer Y to the question asking confirmation. Wait until the message Press D to resume data transfer appears on the live display, then exit from the terminal emulator. After returning to the terminal emulator press D to resume data transfer.

2.17 Loading the cassette rack

2.17.1 Cassette rack

The cassette rack is located inside the door and consists of 16 or 32 shelves, numbered 1 to 16 (32) from the bottom upwards. The maximum capacity is therefore 1536 (3072) samples when using 96-well plates, and 384 (768) samples when using 24-well plates and 6144 (12288) samples when using 384-well plates. Any combination of the two types of plates accepted by your MicroBeta (96 and 24 or 96 and 384) can be used. The type of cassette (384, 96 or 24 position cassette) is recognized automatically by the counter.



2.17.2 Loading

The cassette rack is in its middle position when being loaded. Cassettes should be loaded into the rack with the sample plate upwards and the ID labels towards you. The cassette to be counted first should be inserted on the bottom shelf, numbered with 1, and the next cassettes on the following shelves. Empty shelves between cassettes are allowed, and the first cassette must not necessary be on shelf no. 1 as long as there is no other cassette below it.

After loading, close the door properly.

2.17.3 Operation

The movement of the cassette rack, and cassettes on the transportation system normally occurs automatically without any need for assistance from the user. However, manual operation is also possible. Press O (Operate conveyor) in the Ready or Count state to get the cassette movement functions. Press O (Off) to stop the cassette movement, C (Clear) to clear the conveyor (i.e.

move a cassette from the transportation system to the rack and to move the rack to the middle position), U (Up) and D (Down) to move the rack up and down, and Q (Quit) to return to the Ready state. (MultiCalc must be in terminal mode if you want to do these operations from within it).

NOTE: Do not move the rack up or down when a cassette is in the counting position. Always first clear the conveyor. Re-start the system if the rack and transportation system becomes blocked.

2.18 Luminescence counting

(See chapter 2J.18 for JET information)

2.18.1 Introduction

Luminescence assays come in two main forms; those making use of luminescence that has a short signal and those making use of that which emits a long stable signal. Both can be measured with a liquid scintillation counter, however the short emission assays prove difficult due to the requirements for reagent addition, mixing and temperature control. More recently assays with a more stable luminescence chemistry have been described and marketed. These have been targeted towards the molecular biology laboratory and have utilized both traditional luminometers and photographic film/image analysis to quantify results. With MicroBeta TriLux, luminescence assays designed to be completed on a solid support e.g. nylon membrane, glass fibre or coated plate, and in solution can be directly quantified in a shorter time than with any of the above methods.

Due to the high signal, luminescence reactions can be measured using single PMTs. The traditional microtitration plates are totally opaque (black or white). These plates are counted with the upper tube and a 1450-105 cassette is used. The filters are counted with the upper or lower tube and a 1450-104 filter cassette is used.

A special feature when using MicroBeta as a luminometer is the 1450-466 coloured sealing tape. The saturation of PMTs is prevented and crosstalk between adjacent positions is minimized by cutting the intensity of luminescence light with the 1450-466 coloured sealing tape.

The luminescence units used are LCPS: luminescence counts per second i.e. corrected CPS-values/100. Before counting the actual samples, the detector and hence the LCPS-values should be normalized with a luminescence solution as similar to the actual samples as possible.

The normalization is done first and it is made use of when counting the actual samples.

2.18.2 Preparation of normalization samples (96 and 384-well format)

Make a sample solution which corresponds to the samples to be analyzed, i.e. has the same enzyme and substrate and pipette the same volume as in the samples, into well/membrane G11 (96-well, 1-12 det.), N22 (384-well, 1-6 det.) and M22 (384-well, 12 det.).

An empty background plate is used for background measurement.

The 96-well sample array is as follows, where S1 is the sample solution:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G											S1	
H												

Plate

Close the sample plate with 1450-461 sealing tape or with 1450-466 coloured sealing tape and place on a 1450-105 cassette for counting.

Filter

Enclose the filter in a 1450-432 plastic sample bag and place it on a 1450-104 cassette; use 1450-466 coloured sealing tape between the sample bag and detector.

2.18.3 Preparation of normalization samples (24-well format)

The procedure is the same as with a 96-position sample support, but the normalization sample position is D5 for S1.

	1	2	3	4	5	6
A						
B						
C						
D					S1	

2.18.4 Normalization protocol

Edit the normalization protocol so that it is suitable for luminescence measurements. To do this in the Ready-state press P (P)rotocol then N (N)ormalization protocols.
Select edit (E) and the protocol number.

```
 1 Protocol name:                  Luminescence
 3 Crosstalk correction (Y/N)                 N
 5 Isotope 1:  1)H-3    2)I-125  3)C-14
               4)S-35   5)Cr-51  6)P-32
               7)P-32 Cerenkov   8)Other
               9)Luminescence                 9
      PMT use: 1)Normal  2)Upper   3)Lower    2
10 Counting time [s]                        1.0
12 Precision (2 sigma) [%]                  0.2
30 Printer output:  1)No    2)Short
                    3)Long  4)Programmable    3
32 Display output:  1)No    2)Short
                    3)Long  4)Programmable    3
34 External output: 1)No    2)Short
                    3)Long  4)Programmable    1
36 File output:     1)No    2)Short
                    3)Long  4)Programmable    1
40 Change special features  (Y/N)            Y
41   Isotope activity setting  (Y/N)         N
42   Background sample  (Y/N)                Y
       Background counting time [s]       60.0
43   Half-life correction  (Y/N)             N
45   Use password  (Y/N)                     N
```

Edit the protocol parameters. Select luminescence (9) on line 5, isotope 1, and counting time 1s on (line 11). A background plate is counted only if selected on line 42.

2.18.5 Normalization counting

Start the normalization run in the Ready or Count state using automatic counting and ID codes, or by selecting Nx, where x is the number of the protocol.

2.18.6 Counting the actual samples

Edit a counting protocol:

In the Ready state select P (P)rotocol then C (C)ounting protocols.

Then select edit (E) and protocol number.

On parameter line 2 select 1 (CPM) and on line 3 select the number of the luminescence normalization protocol defined above. On line 42 select Y (Background correction) and on the next line Y (Use normalization background). Edit the other parameters normally.

Start counting in the Ready or Count state using automatic counting and a protocol ID label or start with the protocol number and press Enter.

When counting the samples, the CPS-values are shown on the status display. The stored normalization factors are used for correcting the LCPS-values in the printout. The normalized LCPS-values are shown after counting on the status display.

2.19 Microtitration plate format output

2.19.1 Advantages of plate format

It is often more natural to print out MicroBeta counting results in microtitration plate format because then it is easier to locate the results for a given sample. Plate format also has the advantage that it often reduces the paper consumption in printer output.

2.19.2 Plate format selection

Microtitration plate format can be selected by including the keyword `PLATE` in the programmable output selection of the counting protocol. The user must then also select the printout fields of interest. Each printout field (such as `CCPM1`) will be output as its own table of 6 or 12 columns and 1-8 rows. MicroBeta outputs all the selected tables after the cassette has been counted. The output can contain both normal and plate format output. Items before the `PLATE` word are output in normal format and those after it in plate format.

Note: If you are using MultiCalc Assay protocols you can also make plate maps there. See section 2.22.4.2 and the MultiCalc manual chapter on Plate operation. Plate mapping is done in a MultiCalc Assay protocol as part of the `CODING` section. F6 (`PLATE`) is the softkey to press.

2.19.3 Restrictions

Some restrictions exist, caused mainly by memory requirements:

- Plate format cannot be used if the Repeats parameter is greater than 1.
- Plate format cannot be used if the spectrum plot (symbol `SPECTRA`) has been selected for printer output.
- Plate format should be selected for the Display only if results are filed with UltroTerm. In this case the plate format should come between the data markers #PS and #PE.
- A maximum of 4 different printout fields will be accepted resulting in up to 4 tables.
- If plate format is selected for more than one output medium (display, printer, external, result file) then the printout fields listed with the first occurrence of `PLATE` will be sent to each medium although output selections would list different fields.
- A cassette will be measured totally before the plate tables will be output.

2.19.4 Examples

Example 1 demonstrates the use of table format. In the output selection `PLATE SQP(I) CCPM1 CCPM1%` the keyword `PLATE` selects microtitration plate format. A separate table is created for `SQP(I), CCPM1` and `CCPM1%` values. If the plate is of the 24-well type and all rows (A,B,C,D) have been specified, the resulting tables are full of 4x6 results.

```
Counting protocol no: 33                          Fri 28-June-2000 15:11
Name: PlateTEST1
CPM normalization protocol no:40

Assay: - / Prot:33 / Cass: - / Func: - / Cassette No:1 / Shelf: 3 / 4*6

SQP(I)
          1        2        3        4        5        6
A      373.9    348.5    382.9    352.4    379.5    348.7
B      291.6    219.1    292.2    221.5    288.5    218.1
C      377.9    349.4    377.5    348.6    377.9    346.2
D      290.1    222.4    290.1    220.3    289.3    218.9

CCPM1
          1        2        3        4        5        6
A      89950    89374    91991    91113    92526    91324
B      86066    75547    86783    76144    86622    74871
C      92202    90716    92217    91019    92012    90123
D      86131    76157    86924    75498    86034    75577

CCPM1%
          1        2        3        4        5        6
A       1.2      1.2      1.2      1.2      1.2      1.2
B       1.2      1.3      1.2      1.3      1.2      1.3
C       1.2      1.2      1.2      1.2      1.2      1.2
D       1.2      1.3      1.2      1.3      1.2      1.3

Total count rate: 2066932.1 CCPM    31.05nCi    34.45 kBq
End of assay
```

Example 2 demonstrates how to include replicate statistics with the microtitration plate format output. Here the keyword STAT can be used (see also the example in section 2.29.3.3 Statistics/File output). The printout fields defined after STAT will be output only when a replicate group is ready. With the plate format output this means that the printout defined after STAT will not be represented in table format but as normal output. In addition, results will be output at once - so the statistics will appear in output before the plate format raw data. In Example 2 the output selection: PLATE CCPM1 CCPM2 STAT REPL MEAN1 CV1% MEAN2 CV2% S1 can be interpreted as:

- PLATE : Use microtitration plate format,
- Collect CCPM1 and CCPM2 values into tables and output those tables when the cassette has been measured,
- STAT : Do not collect the following printouts into tables but output them when the replicate mean value is available,
- REPL : Output replicate range (e.g. A01-A03),
- MEAN1 - CV2% : Output these statistical values,
- S1 : Output deviation values (CV1%, CV2%) at the 1-sigma level.

```
Counting protocol no:33                  Fri 28-June-2000  15:22
Name: PlateTEST2
CPM normalization protocol no:40

Assay: - / Prot: 33 / Cass: - / Func: - / Cassette No:1 / Shelf: 3 / 4*6

   REPL      MEAN1      CV1%      MEAN2  CV2%
A01-A03   29560.1      15.5    97524.5   0.8
A04-A06   31609.3      13.5    95978.3   0.8
B01-B03   61304.6      20.6    87538.6   7.9
B04-B06   68693.1      16.7    83309.6   7.9
C01-C03   29861.3      14.4    97421.2   0.7
C04-C06   32664.4      14.0    96547.0   0.7
D01-D03   62968.0      20.3    89112.1   6.8
D04-D06   69314.6      16.4    83678.5   8.3

CCPM1
         1        2        3        4        5        6
A    27775    34766    26137    33792    26680    34354
B    53926    75863    54123    76060    55490    74528
C    27296    34828    27459    35104    27400    35487
D    55602    77735    55565    75730    56205    76007

CCPM2
         1        2        3        4        5        6
A    97465    96759    98349    96360    96441    95132
B    91277    79595    91742    80547    90815    78565
C    98128    96844    97291    96366    97342    95932
D    92820    82171    92343    79503    91738    79793

Total count rate 1:  1157926.1 CCPM    521.59 nCi    19.30 kBq
                 2:  2193329.2 CCPM    987.99 nCi    36.56 kBq
End of assay
```

2.20 Micro-volume LSC

2.20.1 OptiPhase™ SuperMix cocktail

In micro-volume counting very small volumes of sample and cocktail have to be mixed. Traditional cocktails are not well suited for this. A cocktail specifically for micro-volume counting has been produced, it is called OptiPhase SuperMix. Aqueous samples can be mixed with a minimum of shaking, and sample loading can be greater than 1 to 1, see the table below. The values are in ml per 10 ml of OptiPhase SuperMix cocktail at different temperatures.

Solute	15°C	20°C	30°C
Deionized water	7.50	25.00	24.50
0.01M PSB	18.50	19.50	16.50
40% sucrose	13.00	10.50	10.00
1.0M sodium chloride	11.00	9.50	7.50
0.5M sodium chloride	15.50	13.00	10.50
0.1M sodium hydroxide	17.00	17.00	17.00
0.05M Tris HCL	19.50	20.00	19.00
0.1M hydrochloric acid	16.50	15.50	14.00
0.25M ammonium sulphate	14.00	13.50	11.50
0.1M ammonium sulphate	16.00	14.50	13.00
Krebs-Ringer original buffer	16.75	16.75	15.50

The solvent used, di-isopropylnaphthalene (DIN), is in all OptiPhase HiSafe cocktails including OptiPhase SuperMix. DIN is not aggressive, so it does not attack the majority of commonly used plastics (e.g. polyethylene, polypropylene, polycarbonate, polyester and PVC) unlike other LSC solvents. This makes it ideal for use with microtitration plates.

2.20.2 Safety aspects

The toxicological and physico-chemical properties of DIN have been extensively studied (see the booklet Di-isopropylnaphthalene - a new solvent for LSC, 1989 available on request). The conclusion of the study was that DIN is classified as a non-hazardous substance within the meaning of the EEC-Directive of the 18th September 1979 (79/831/EEC). DIN is not subject to compulsory labelling as laid down in those regulations which are effective in the European Community. In accordance with UN and EEC International Transportation Regulations, DIN is not required to be labelled as flammable or hazardous because its flash point exceeds 65°C (it is in fact 148°C). A biodegradability study of OptiPhase HiSafe II will also be found in the above mentioned booklet with the conclusion The test compound, OptiPhase HiSafe II was found to be readily biodegradable by the ISO 7827-1984 (E) method.

OptiPhase SuperMix, belonging to the OptiPhase HiSafe family, shares these same features which thus greatly simplifies the requirements involved in its transportation and disposal as well as showing it to be both safe to use and environmentally friendly. In many countries it can be disposed of via the drains because of its biodegradability. It is also possible to use a removable adhesive seal for the microtitration plates so that liquid and solid waste can, if necessary, be separated and thus disposal costs reduced. However, it is essential to consult with your Radiological Safety officer and Institute Administrator before entering into any particular course of action.

2.20.3 Filling wells

Do not overfill wells. In the case of the flexible MicroBeta 96-well plate the maximum total volume of liquid should not be much above 200 μL; rigid plates have a capacity of 350 μL. Splashes of scintillant on the plate should be removed with a tissue prior to applying the adhesive seal.

2.20.4 Preparation of a quench series

If you are using constantly quenched samples then the preparation of a quench series is not necessary. However MicroBeta also offers you the possibility to handle variably quenched samples. In such a case it is necessary to prepare a quench standard series so that the quench correction necessary to calculate the final DPM values can be made.

The preparation of six samples is adequate for a quench series, although up to 12 samples can be used with MicroBeta. Each sample should contain the same amount of radioactivity but the amount of quenching agent in the cocktail should increase incrementally so that the first sample has no quenching agent and the last the greatest amount. The range of the quench standards should be such that it covers the expected quench range of the unknown samples and also that it allows the quench curve to be well defined by the curve fitting program.

2.20.5 96-well plates

Prepare a series of quenched OptiPhase SuperMix samples. E.g. dispense into 5 ml samples of OptiPhase SuperMix the following amounts of carbon tetrachloride: 0, 10, 30, 50, 75 and 100 μL. Mix these samples.

Next dispense the activity to wells A1 to A6, G11 and G12, then dispense 200 μL of the quenched cocktail to each well. Seal the plate and count it as described in chapter 2.11.

The activity used should be e.g. 20 μL of tritiated water or some other suitable source giving about 200 000 DPM per well so that the counting time required to get an acceptable precision (e.g. 0.5%) can be about 1 min. If you do not mind using a longer counting time you can use lower activities.

Note: The total time for a standardization with 200 000 DPM activity would be less than 20 mins whereas for 20 000 DPM it would be 3 hrs to get the same precision.

2.20.6 Chromium release

Specific cytotoxicity can be measured with microvolume samples by counting the supernatant from the chromium release. Add 25 µL of supernatant to 175 µL of OptiPhase SuperMix cocktail. The crosstalk between wells is of the order of 0.06% when using the printed microtitration plates. Use a counting window setting of 5 - 170.

2.20.7 Microvolume LSC and chromatography

A chromatography eluent gradient with varying ionic strength can be collected in 96-well plates for evaluation in MicroBeta. The different aliquots will have different levels of quenching and hence different SQP(I) values. The SQP(I) value is then used to obtain the efficiency and hence the DPM for the sample.

In order to automate dispensing to a 96-well plate you can use a device such as the Labsystems Multidrop liquid dispenser.

2.20.8 Scintillation Proximity Assay (SPA)

2.20.8.1 Introduction

The scintillation proximity assay developed by Amersham is an application for which MicroBeta with the ParaLux counting feature (see section 2.20.9 below) is very well suited.

In SPA, fluomicrospheres coated with receptors or acceptor molecules are used for binding the ligand to be assayed. These fluomicrospheres also constitute the scintillation medium. Low energy electrons e.g. tritium beta particles or I-125 Auger electrons, from the radioactively labelled bound ligand cause light emission from the fluomicrospheres. Labelled, unbound ligand does not cause light emission because of the dilute concentrations used in bioassays where the distances between the unbound ligand and the fluomicrospheres is greater than the maximum range of the low energy electrons; they are absorbed in the aqueous medium. Consequently there is no need to separate the unbound ligand. The whole assay is performed in the microtitration well and the result counted in a beta scintillation counter such as MicroBeta.

2.20.8.2 Colour quenching and SPA

If the SPA involves coloured solutions then there may be a need to make a colour quench correction. Amersham provides colour quench kits for use with MicroBeta. These are:

^{3}H SPA PVT colour quench and calibration kit code TRKQ 7080.

^{125}I SPA PVT colour quench kit code RPAQ 4030.

(Note: for more details of DPM standardization procedure with MicroBeta see chapter 2.12 DPM Standardization).

Load the standardization plate into a cassette with a STD label in the FUNC position and a suitable standardization protocol number and load this standardization cassette into MicroBeta.

Edit the standardization protocol and set the standardization parameters shown overleaf. In particular select the SPA mode (mode 2). This automatically selects ParaLux counting.

Run the standardization. The results will be saved under the standardization number marked on the cassette.

When you come to run the actual SPA, set the counting protocol for DPM and give the standardization protocol number selected above. Other parameters should be set according to the instructions in your SPA kit insert.

```
 2 Sample type: 1)Normal 2)SPA                -> 2
 5 Isotope 1:  1)H-3    2)I-125  3)C-14
               4)S-35   5)Cr-51  6)P-32
               7)P-32 Cerenkov   8)Other      -> 1 (H-3) or 2 (I-125)
     ParaLux  (Y/N)                           -> Y
       ParaLux mode:  1) High efficiency
                      2) Low background       -> 2
       Discriminator channel                  -> 150
     PMT use:  1)Normal 2)Upper  3)Lower      ->
     Window 1: (default)                      ->
10 Counting time for crosstalk [s]            -> 600 (sec)
. . .
20 Number of standards      •                 -> 10
21 Isotope 1 activity [DPM]                   -> x (where x is the activity
                                                   of a sample in DPM)
. . .
The remaining parameters can be selected to be default.
```

2.20.9 ParaLux counting

2.20.9.1 What is ParaLux counting?

In the case of scintillating samples e.g. SPA-beads, that are settled on the bottom of a well, the scintillation light from the sample, has to travel a longer distance through the sample solution in order to reach the upper photomultiplier tube. The light reaching the lower PMT has to travel a shorter distance and it does not have to pass through much sample solution. This

causes a scintillation light detection asymmetry because events may be detected in the lower tube which are not detected in the upper tube because of quenching. This asymmetry is most pronounced with heavily colour-quenched samples.

In the normal counting mode, each pair of PMTs works in coincidence, which means that both must detect the scintillation event for it to be counted. However, in the case described above, there are true scintillation events from the sample which are detected by the lower PMT but which are not in coincidence with any event detected by the upper tube. These events would normally be rejected by the coincidence system.

In ParaLux counting mode, both the coincidence events and the non-coincidence events in the lower tube are counted. In addition a new quench parameter called AQP(I) (Asymmetric Quench Parameter) is used. This parameter is derived from the difference between the coincidence counts and the counts observed in the bottom PM tube. In other words it establishes a relation between quench and efficiency.

2.20.9.2 Counting modes

ParaLux counting is set in the protocol at the same time as the isotope is selected, see chapter 2.25 Protocols. ParaLux counting has two modes: Low background mode (default) and High efficiency mode. The difference between them is as follows.

In the Low background mode, the count rate is calculated from just the coincidence events. In the High efficiency mode the count rate is calculated from the coincidence events plus the events not in coincidence that are detected in the lower tube. However these latter events are only registered for pulses that come in channels exceeding the discrimination channel set for the MCA. You can set this discriminator channel level (the default is 150). Both modes use AQP(I) as the quench parameter.

Both modes give improved counting performance because of the use of the Asymmetric quench parameter. The High efficiency mode gives a higher efficiency but also a higher background than the other mode.

2.21 Monitors and flags

2.21.1 Functions of monitors and flags

Monitors are used to check the quality of samples and flags to notify of e.g. poor sample quality or that counting has been terminated. They are selected in the programmable output in protocol editor (see chapter 2.25 Protocols). The flag is included also in the short and long outputs and the DPM monitor in DPM counting. At the end of this chapter is a list of monitors and flags with their output headings and possible values.

2.21.2 Statistics monitor

The purpose of this monitor is to warn of unacceptable count rate variation in a sample. The sample count rate in different periods of the counting time is measured to determine the sample count rate variation. The variation must be within the range expected from the statistical nature of radioactive decay. If the variation is outside the range, due to e.g. chemiluminescence, the sample is recounted. A maximum of two recounts are made before the results from the last recount are output.

The statistics monitor is not active unless it has been selected in at least one programmable output.

2.21.3 DPM out-of-range monitor

This monitor evaluates quench levels of samples in DPM counting and compares these with corresponding quench levels in the standardization. This is done by measuring SQP(I) for a sample. If it is not within the standardization range the monitor gives a DPM out of range message.

The DPM out-of-range monitor is included in the short and long outputs in DPM counting. It is no longer in use if it has been removed from the outputs.

2.21.4 LCPS out-of-range monitor

This monitor evaluates the light intensity of samples in luminescence counting to inform of possible counter saturation. The monitor is set to HIGH if counter saturation is detected. The LCPS out-of-range monitor is included in the short and long outputs in luminescence counting. It cannot be used with the luminescence scaler board.

2.21.5 Termination flag

Counting is normally terminated when the specified counting time is reached or when the user stops it, but in some cases it can be interrupted earlier. Precision, selected on line 12 in the protocol editor, is such a case (see chapters 2.25 Protocols and 2.6 Counting control). The termination flag is set to 'PREC' if the precision value has been reached. The counting stops if the precision has been reached in all detectors.

Another case that terminates counting is maximum counts or 'hot sample'. If the count value in any channel in any detector exceeds a certain value, usually 50000 counts, the counting stops and the flag for the particular sample is set to 'MAX'. This prevents MCA channels from overflowing.

The flag is set to 'INT' if the user interrupts the counting by selecting Off, Next position or Next protocol.

Item name	**Heading**	**Value**	**Explanation**
Statistics monitor	STM	.	No statistical variation detected
		REC1	No variation after one recount
		REC2	No variation after two recounts
		ERR	Still variation after two recounts
DPM out of range monitor	DPMM	.	OK, no out of range detected
		OUT	Out of DPM range
LCPS out of range monitor	LM	.	OK, no out of range detected
		HIGH	Counter saturation detected
		?	Luminescence scaler is used (saturation cannot be detected)
Termination flag	FLAG	.	Normal termination time reached
		PREC	Precision reached
		MAX	Hot sample (more than 50 000 counts/channel)
		INT	Counting interrupted by user

2.22 MultiCalc operation

2.22.1 Introduction

MultiCalc is developed from RiaCalc, the famous immunodiagnostic software package with nearly 10 000 users in hospitals and laboratories all over the world. In contrast to RiaCalc, the MultiCalc software can be customized to different labelling techniques, it can handle assays in both sequential and plate format and it supports PC networks. MultiCalc allows result evaluation, screening, microtitration plate format calculations with free replicate placing, a wide range of quality control operations, mainframe communication etc.

MultiCalc runs on any IBM PC or compatible. Graphics supported are CGA, EGA, MCGA and VGA, both colour and b/w.

The contact between MultiCalc and MicroBeta is by means of a special communication protocol.

MultiCalc is operated by softkeys. It assigns different functions to the ten function keys F1 to F10 according to the operating level it is on. The starting set of softkey functions is called the Main menu and is what you get when MultiCalc has been first started up. To get to the main menu from any other level you have to press the F9 key one or more times plus answer any questions about exiting and saving that the program may ask you on the way.

What follows here is a description of some of the features which are especially relevant to the operation of MultiCalc with MicroBeta. The softkey sequences described begin with the main menu referred to above.

2.22.2 Installation and startup

The installation procedure is as follows.

a) When you install MultiCalc on your PC you will be asked amongst other questions to select the Technology and the Counter. Technology describes the counting process to be used. In this case it is beta counting so you must select BETA. Counter is the type of counter MultiCalc is to be working with. You must select PLATEV3. You will be asked to load a Disk with communication drivers. Drivers are instrument version specific so please use the instrument program disk at this point. You can select more than one type of technology and counter at the same time. Complete the rest of the MultiCalc installation as described in the MultiCalc User Manual.

If MultiCalc is already installed in the PC but not for MicroBeta then you must go through a similar procedure to that described above but you select Setup only rather than Installation from the main installation menu.

(b) Start MultiCalc.

(c) Press F1 (= COUNTER) to get to counter control.

(d) Move the cursor to PLATEV3 in the counter list.

(e) Press F5 (= INSTALL) to install 1450 MicroBeta for MultiCalc. In step a), you were adding MicroBeta to the list of possible counters to be operated with MultiCalc. Now with this instruction you are telling which counter you are actually going to work with.

If MultiCalc cannot get MicroBeta into the Ready state it will ask you to press F3 to get into the terminal mode.

Then press F3 and try to get MicroBeta into the Ready state. If communication does not seem to work then see chapter 2.31.4.11 Terminal emulators/GenTerm/ Trouble shooting (1) about how to try to fix the problem. You can also try to press the Esc-key several times.

(f) At the end of installation answer YES to see instructions about MultiCalc/MicroBeta use.

Note: Steps (b) through (f) should be executed when:

- MultiCalc is connected for the first time to MicroBeta

- MicroBeta has been used in another environment (such as with UltroTerm)

- There seems to be some problems

For more details about MultiCalc installation see the MultiCalc User Manual, Installation.

2.22.3 MultiCalc terminal mode

Sometimes you must use terminal mode, e.g. when changing counting, standardization or normalization parameters. MultiCalc allows you to operate in terminal mode as mentioned in the previous section. The type of terminal emulation used by MultiCalc is VT52. I.e. when MultiCalc is in terminal mode MicroBeta sees it as if it was a VT52 type terminal. MultiCalc must therefore be selected as terminal emulation in the MicroBeta System mode parameter Ter(m)inal (see chapter 2.30.10 System/Ter(m)inal).

To get to terminal mode select F1 (=COUNTER) from the MultiCalc main menu, followed by F3 (= TERMINAL). In this mode MicroBeta can be used as with any other terminal emulator. Press F9 (EXIT) to exit from terminal mode and go back to the normal MultiCalc counter mode.

Press F10 (HELP) to get assistance in almost every situation.

2.22.4 Assay protocols

The main difference between operating MicroBeta with a terminal emulator or operating it with MultiCalc in counter mode is in terms of the data handling abilities. In the former case everything is determined by the MicroBeta counting protocol which is what is described in most of the other chapters in part 2 of this Instrument Manual. Counting protocols are normal liquid scintillation counting protocols containing parameters which control counting (see chapter 2.25 Protocols/Parameters in a counting protocol). These are used (e.g. edited) without MultiCalc or in the terminal mode of MultiCalc. Assays with only counting protocols can also be started from MultiCalc, but the results are not evaluated by MultiCalc. The PROT-ID on the cassettes identifies assays using Counting protocols.

If, however, you are using MultiCalc counter mode then you work with MultiCalc assay protocols. Assay protocols are connected to counting protocols. An Assay protocol contains protocol number, name, counting time and precision, plate coding and outputs. Other parameters, such as isotopes, CPM/DPM mode, corrections, etc., are retrieved from a corresponding Counting protocol, which is selected in the Assay protocol editor.

Assay protocols can only be edited in MultiCalc (see Editing protocols below). They are stored in both MultiCalc and in the counter. New assay protocols and updates are transferred to MicroBeta by using the PARAMETERS function. To get this press F1(= COUNTER) followed by F2 (= PARAMETERS).

The results are sent to MultiCalc in raw form for handling according to the instructions given in the MultiCalc assay protocol. The great range of data handling possibilities available with MultiCalc are described in the User guide to MultiCalc functions and other manuals. See especially there the chapters on Protocols, Files and Quality control.

Assays using Assay protocols must always be started from MultiCalc. Samples using Assay protocols are identified by an ASSAY label on the first cassette in a batch. They can have numbers from 1 to 99.

2.22.5 Using MicroBeta with MultiCalc

2.22.5.1 Editing protocols

MicroBeta controlled counting protocols, i.e. Counting, CPM normalization and DPM standardization protocols, must be edited in terminal mode. Select COUNTER (F1) in MultiCalc's main menu (press F9 one or several times to get to this level), move the cursor to PLATEV3 in counter mode and select TERMINAL (F3). Protocols can then be edited as described in chapter 2.25 Protocols.

Assay protocols, which are controlled by MultiCalc, must be edited in MultiCalc. Select PROTOCOLS (F4) in MultiCalc's main menu and a list of Assay protocols is displayed. Select CREATE (F2) to create a new protocol. Enter name, protocol ID (or number), and select BETA as the technology and RIA, IRMA or RATIO as assay type.

To edit an existing protocol, move the cursor to the protocol line and press Enter. If the protocol list is more than one page long the next page can be displayed by pressing the Page down-key (usually marked PgDn). Similar, the Page up-key (PgUp) is used for scrolling one page up. Press the Home-key to get the first protocol and the End-key to get the last protocol in the list. A protocol can also be selected by giving the beginning of its name.

After pressing Enter, the protocol with its parameters is displayed. Use the cursor keys to move up and down, and press Enter to change the value on a particular line. The alternatives, if any, are displayed as softkeys (F1 - F8), otherwise the value is given by typing it.

The beginning of the protocol looks as follows:

```
01 DUAL ASSAY
02 COUNTING TIME, MAX COUNTS
03 MEASURING PARAMETERS
```

Select dual (press F1) or single (F2) label results handling. Note that dual assay here means the way that MultiCalc handles the results. This is not the same as the counter's dual label. Instead, dual label counting is selected by using a dual label counting protocol.

Enter counting time in seconds and, separated by a space, the Max counts or precision value. Note that this is expressed as 1-sigma percent while the precision in the counter is expressed as 2-sigma percent.

Select the isotope(s) on line 03 MEASURING PARAMETERS using one of the softkeys F1 to F8. The isotope names can also be typed in. The Counting protocol to be used corresponds to the selected isotope as shown in the table following:

Key	Isotope	Counting protocol
F1	^{3}H	91
F2	^{125}I	92
F3	^{14}C	93
F4	^{35}S	97
F5	^{51}Cr	94
F6	^{32}P	95
F7	$^{3}H/^{14}C$	96
F8	^{32}P Cerenkov	98

The Counting protocols 91 to 98 are factory made. They may not be deleted and the isotopes may not be changed. They are connected to factory made CPM normalization protocols with the same numbering (91-98).

Note: No CPM normalization or DPM standardization is made at the factory, so you must prepare suitable normalizations or standardizations for the factory made counting protocols.

The Counting protocol number can also be typed instead of giving the isotope. Please check that this protocol exists and that it has the right isotope(s) and counting mode. If the protocol does not exist the default protocol (number 0) will be used.

Examples of selections on line 03:

```
H3        - Counting protocol 91 is used.
H3/C14    - Dual label, Counting protocol 96 (3H/14C) is used.
18        - Counting protocol 18 is used (can be single or dual label)
```

If you are using a newer version of MultiCalc you will first be asked for the counter instead of the isotope. Press softkey PLATEV3. A list of counting protocols will be displayed and the isotopes available will be selectable via softkeys. You can now either select a factory made counting protocol by pressing an isotope softkey or select a protocol from the list. Scroll the list by the up and down arrow keys (or PageUp/PageDown for faster scrolling) and make the selection by pressing Enter.

Note: If you have a newer version of MultiCalc but do not get the counting protocol list, try to install the counter again. Press F5 (=INSTALL) in the counter mode and the counter will send the protocol index to MultiCalc.

See also the Introduction to MultiCalc operation manual, the module on Protocol operations.

2.22.5.2 Plate coding

Plate coding is accessed by pressing F1 (CODING) (See the MultiCalc User Manual Plate operation).

Plate coding requires the replicate number and the sample type to be set. Press F7 to increase or F8 to decrease the replicate number. Press F1 to F4 to select the sample type. Then press Enter to get a new line for the next replicate type.

The PLATE-key (F6) allows a sample layout to be defined directly onto the plate. After pressing F6, a plate is displayed showing the different codes in different wells. Use the cursor keys to move around the plate. Select EDIT (F1) to edit existing layouts or CREATE (F2) to define a layout of your own. See the MultiCalc manual for how to make the plate layout.

Press F9 to exit the plate coding.

Again, press F9 to exit from protocol editor. Select F1 to save changes to the protocol or F2 to exit without saving.

2.22.5.3 Sending assay protocols to the counter

Assay protocols must be stored in both MultiCalc and the counter. After exiting from the editor the protocol will only be stored in MultiCalc and it must therefore be sent to the counter. Go to the counter control (press F9 and F1) and select PARAMETERS (F2). Press F2 to send every Assay protocol or move to the protocol name and press Enter to send a particular protocol.

If you want to check what assay protocols have been sent to the counter, you can use commands P and M in the MicroBeta Ready state. (This comes from the command list items (P)rotocols/(M)ultiCalc assay protocol).

There are three functions you can select from:

Show - displays the contents of an assay protocol

Print - prints out the contents of an assay protocol

List - prints the list of assay protocols

2.22.5.4 CPM normalizations and DPM standardizations

Normalizations and standardizations are made in the same way as when using MicroBeta without MultiCalc (see chapters 2.23 Normalization and 2.12 DPM standardization). Go to the counter control in MultiCalc and select TERMINAL (F3). Select normalization or

standardization protocol in the Ready state to edit the protocol. Start the counting as normal from the Ready state.

Normalizations and standardizations can also be started from MultiCalc. Check that a NORM or STD label is in the FUNC field of the cassette. Select PLATEV3 in the counter control and press Enter to start counting.

NOTE! The results files from the normalizations and standardizations will not be evaluated by MultiCalc!

2.22.5.5 Counting unknown samples

Unknown samples can be counted by using MicroBeta controlled protocols (Counting protocols) or MultiCalc controlled protocols (Assay protocols). The type of protocol is selected by putting an ID label with protocol number in the PROT field of the cassette for Counting protocol assays. When using Assay protocols the ID label is attached to the ASSAY field.

The counting of samples using Counting protocols is started as when using MicroBeta without MultiCalc, i.e. select Automatic counting in the Ready state. It can also be started from MultiCalc by pressing F1 (=COUNTER) and then selecting PLATEV3 and pressing Enter. The live data and results are displayed on screen and results are sent to each output medium as selected in the protocol. Printer outputs are sent to the printer connected to the PC if Print through terminal is selected in System/Ter(m)inal. Results to file are sent through MultiCalc to the appropriate disk drive on the PC.

NOTE! The results from assays using counting protocols will not be evaluated by MultiCalc!

The counting of samples using Assay protocols may only be started from MultiCalc. Check that the protocol number label is in the ASSAY field of the first cassette in a batch. Select PLATEV3 in the counter control and press Enter to start counting. Live data can be displayed by pressing F4 (=LIVE). The results of each sample are displayed in plate format if plate coding has been used. The results are then stored in MultiCalc for further evaluations (see Stored files and Result files in the MultiCalc manual).

2.22.5.6 Communication protocol

See MultiCalc User Manual Communication protocols.

To change the RS232C port, change only lines from 02 COMM.PORT to 07 COMM.MODE.

2.22.5.7 Communication problems

See chapter 2.31.4.11 Terminal emulation/GenTerm/ Trouble-shooting for information about how to solve possible communication problems.

2.22.5.8 Printer connection

MultiCalc prints results to the printer connected to the terminal PC with a standard Centronics cable. In terminal mode MicroBeta controlled assays direct printing also to the same printer.

2.22.5.9 Exit to MS-DOS

In the main menu select first LEVELS (F7) then MS-DOS (F8). See the MultiCalc User Manual for details of other menu operations.

2.23 Normalization

(See chapter 2J.23 for JET information)

2.23.1 What is normalization?

MicroBeta has 1, 2, 3, 6 or 12 detectors allowing it to count the respective number of samples simultaneously. In order for the results from each detector to be equivalent, irrespective of small variations in efficiency and background between detectors, it is necessary to determine the relative efficiency and background of each detector and then correct for it. This is called normalization.

Normalization is done by measuring an optional background plate followed by one or two standard samples with defined activity or CPM in each detector. Once CPMs have been measured the relative efficiencies can be calculated. The efficiency of the detector giving the highest count rate is then defined to be one and the other detector efficiencies are expressed as a fraction of this value. These fractions are called efficiency coefficients.

If isotope activity is given, then absolute efficiencies are calculated by dividing the count rates by the activity. In this way, sample quench and detector absolute efficiencies can be corrected for.

When measuring a sample with a particular detector, the CPM of the sample is corrected by dividing the CPM by the efficiency coefficient. In dual label counting, corrections are made using dual label correction formulae (see 3.3 Calculation methods).

When normalization is done the results are stored with the normalization protocol. The normalization data can be used by one or several protocols and is selected when editing the protocol (see 2.25 Protocols).

2.23.2 When is normalization necessary?

Each counting or assay protocol requires normalization data to be stored before it can calculate corrected CPMs for the samples counted. A fresh normalization must be done when a new isotope is counted or when counting features, such as isotope or window, are changed. When a new type of microtitration plate is used, crosstalk normalization may be necessary.

2.23.3 Editing normalization parameters

Normalization parameters are stored in normalization protocols. These parameters are shown in the figure. There can be a maximum of 100 normalization protocols, numbered from 0 to 99. Protocol number 0 is the default protocol.

```
Line (1..46, type / to exit, ? for help)      1 ->

 1 Protocol name:
   ->
 3 Crosstalk correction (Y/N)                 N ->
 4 Number of labels:  1)Single  2)Dual        1 ->
 5 Isotope 1:  1)H-3    2)I-125  3)C-14
               4)S-35   5)Cr-51  6)P-32
               7)P-32 Cerenkov   8)Other
               9)Luminescence                 1 ->
     ParaLux  (Y/N)                           N ->
     PMT use:  1)Normal 2)Upper  3)Lower      1 ->
     Window 1:                          5- 360
10 Counting time for crosstalk [s]          60.0 ->
12 Precision (2 sigma) [%]                   0.2 ->
30 Printer output:  1)No    2)Short
                    3)Long  4)Programmable    3 ->
32 Display output:  1)No    2)Short
                    3)Long  4)Programmable    3 ->
34 External output: 1)No    2)Short
                    3)Long  4)Programmable    1 ->
36 File output:     1)No    2)Short
                    3)Long  4)Programmable    1 ->
40 Change special features  (Y/N)            Y ->
41   Isotope activity setting  (Y/N)         Y ->
       Isotope 1 activity (DPM)        200000.0 ->180000
42   Background sample  (Y/N)                N ->
43   Half-life correction  (Y/N)             N ->
44   Chemiluminescence correction  (Y/N)     N ->
45   Use password  (Y/N)                     N ->
46   Special plate: 1)Wallac  2)BP filter
                    3)Other                  1 ->
```

Protocols 91 to 98 inclusive are made at the factory for isotopes ^{3}H, ^{125}I, ^{14}C, ^{51}Cr, ^{32}P, dual label ^{3}H/^{14}C, ^{35}S, and ^{32}P Cerenkov respectively. These may not be deleted because they are connected to the corresponding counting protocols. Note that no normalization is done for those protocols. You must use these protocols to do the normalization before they can be used for counting unknowns.

Press P and N in the Ready state to choose the normalization protocol state. The protocols can then be edited, copied, deleted, printed and displayed. Press F to get default parameters and E to edit a protocol. C is used for copying a protocol including normalization data and D for deleting a protocol.

Note: the normalization data will be destroyed when editing an old normalization protocol. The normalization must therefore be done again for this protocol. Only the protocol name and password can be edited without destroying normalization data.

See chapter 2.25 Protocols for further details on normalization parameters and editing.

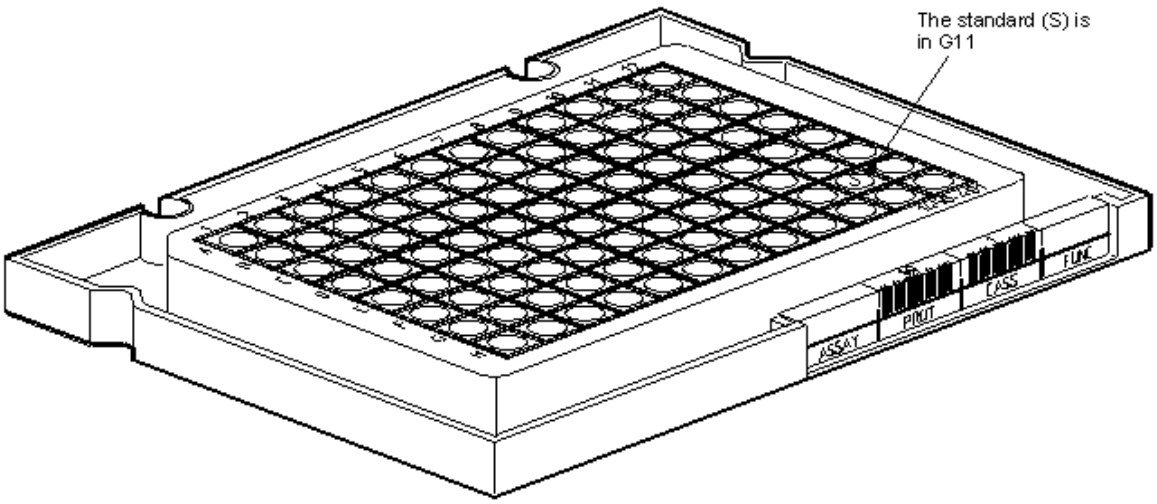
2.23.4 Preparing the normalization samples

Depending on which type of MicroBeta you have it is supplied from the factory with:

(for 1-6 detector instruments with 24 or 96-well capability) a 24-well normalization sample plate containing standards for ^{3}H and ^{14}C. The ^{3}H standard is in D5 and the ^{14}C standard is in D6.

(for 12 detector instruments with 96 or 384-well capability) a 96-well normalization sample plate containing standards for ^{3}H and ^{14}C. The ^{3}H standard is in G10 and the ^{14}C standard is in G12.

(for other configurations of MicroBeta special arrangements are made).



Sample position in a normalization plate

The sample positions and the activities for the standards are shown on a label on the plate. The activity values can be inserted into the normalization protocol.

Be careful that the samples are in the correct positions when making your own normalization plates. The positions cannot be changed by modifying protocol parameters. In 24-well plates the standard for isotope 1 is in D5 and the standard for isotope 2 is in D6. Isotope 1 is the lower energy isotope in dual label counting. In single label counting the standard is in the isotope 1 position, i.e. D5, with one exception:

the standard for ^{14}C must be in the isotope 2 position in single label counting with 24-well plates.

This is because the factory made normalization plate could be used for both single label ^{3}H and ^{14}C normalizations and dual label ^{3}H / ^{14}C normalizations.

For best results, 96-well plates should be used as normalization plates if 96-well sample plates are to be counted in a 1-6 detector counter. The isotope 1 standard should be in G11 and the isotope 2 standard in H12.

See 2.7.2 Crosstalk CPM normalization for crosstalk sample positions and 2.18 for luminescence sample positions.

If background sample is selected on line 42, then thermal backgrounds for count subtraction are measured automatically. An empty background cassette must be the first normalization cassette followed by the normalization cassette with standards.

See the table below for sample positions in normalization plates:

Sample position in a normalization plate						
	24-well	96-well	96-well		384-well	384-well
	(1-6 det.)	(1-6 det.)	(12 det.)		(1-6 det.)	(12 det.)
			Single	Dual		
Isotope 1 standard	D5	G11	G11	G10	N22	M22
Isotope 2 standard	D6	H12		G12		
^{14}C single label	D6					

2.23.5 Normalization procedure

First place the empty background plate in a cassette and then plate the plate with the normalization samples in a cassette which is identified with the corresponding normalization protocol number in the protocol number area and with a NORM label in the function code area. Insert the cassette into the rack and close the door. Press either A (Automatic counting), or

Nxx, where xx is the normalization protocol number, in the Ready or Count state to start counting. If Nxx is selected the program asks:

```
Do you want to continue counting after normalization? (Y/N)->
```

If Y (Yes) is selected, the counting continues after counting the normalization plate, if N (No) is selected the counting stops after the normalization. If normalization has already been done for the protocol then the program asks first:

```
This protocol contains normalization data.
Continue? (Y/N) ->
```

This is to prevent accidental overwriting of normalization data.

If automatic counting is selected the normalization cassette can be placed anywhere in the rack. The NORM label is needed in this case.

The counting results for the normalization are output as specified on lines 30 - 37 in the normalization protocol, and after them the background and detector efficiency values are given.

Note! Normalization and unknown samples should have the same quench level in dual label counting.

2.23.6 Preset normalizations

There are four preset normalizations available in MicroBeta:

Normalization protocol 1 contains a ^{3}H normalization using ScintiStrip plate 1450-419, dry, 200 µL volume with crosstalk correction.

Normalization protocol 2 is the same as 1 but using ^{125}I.

Normalization protocol 3 contains a generally used ^{3}H normalization.

Normalization protocol 4 contains a generally used ^{14}C normalization.

2.24 P-32 Dot blot quantification

Assays utilizing the ^{32}P label and 96-format filtermats, e.g. dot blot hybridizations can be detected with MicroBeta using the ^{32}P cassette (1450-118). Dried sample membranes can be counted directly in the cassette, while wet membranes need to be counted in a sample bag, to avoid any contamination. The counting method does not include any scintillator addition to the actual filter and thus allows re-probing of the samples. When ^{32}P samples are measured with the 1450-118 cassette the obtained results are quantitative. The ^{32}P counting efficiency is around 70% and background less than 10 CPM. Crosstalk values less than 0.05 % are achieved.

The cassette consists of a cassette body and a lid. In the cassette body there is a steel plate, which is the same size as the filtermat. The filtermat is aligned on the steel plate according to the edges and the lid is clicked on the filtermat, so that the small hole is in the upper left corner, the A1-corner.

To count the ^{32}P cassette in MicroBeta, choose other isotope, upper PMT usage and window 350-1024 in your normalization or counting protocol. No crosstalk correction is needed. If you use Windows workstation you can use the parameter ^{32}P cassette 8 by 12 for ^{32}P membranes or you can set up a new parameter with the settings previously mentioned.

2.25 Protocols

(See chapter 2J.25 for JET information)

2.25.1 Four types of protocols

A protocol is a set of parameters which control the counting of samples. There are four types:

- CPM normalization protocol
- DPM standardization protocol
- MicroBeta Counting protocol
- MultiCalc Assay protocol

A CPM normalization protocol contains parameters used when doing normalization (see chapter 2.23 Normalization) and a DPM standardization protocol contains parameters for standardization (see chapter 2.12 DPM Standardization). A MicroBeta counting protocol is used when counting unknown samples. An assay protocol is the corresponding protocol in MultiCalc (see MultiCalc). A counting protocol uses either a normalization protocol (in CPM counting) or a standardization protocol (in DPM counting) to get certain parameters, e.g. isotopes and window settings. Assay protocols are connected to counting protocols, from which they get both basic parameters, such as isotopes and counting mode, and special features, such as background and half-life corrections.

The maximum number of protocols for each type is 100 (0 to 99). Number 0 is the default protocol, which is used if no protocol number is specified.

Counting protocol number 99 is a so-called GLP (Good Laboratory Practice) protocol. Count the normalization sample plate supplied with the instrument using this protocol. Do this from time to time to make sure that the instrument produces consistent results. The background, ^{3}H and ^{14}C samples are counted with all detectors so background level, CPM values and quench parameter values can easily be compared. See chapter 3.2 Routine maintenance for more details.

In this chapter only MicroBeta protocols are described. For further information about assay protocols see chapter 2.22 MultiCalc and the MultiCalc User manual Protocols.

2.21.2 Listing protocols

To list or edit protocols, press P and then C for counting protocols, N for normalization protocols or S for standardization protocols in the Ready state. To select the MultiCalc assay functions as described in chapter 2.22 press M.

To display the protocol list, in the Protocols menu press S (Show). You will see a two column list of protocols containing protocol number, name (if given) and isotopes. The list of the counting protocols also contains counting mode (CPM or DPM) and the number for the normalization or standardization protocol used. The lists of the normalization and standardization protocols have a column containing the date when the normalization or standardization has been done. An equals (=) character indicates that crosstalk correction is used.

If there are more protocols than there is space for on the display, press Enter to get the next page of protocols. When you have found the one you want to look at, type the number of that protocol and press Enter.

Type L (List) to printout the protocol list and P (Print) followed by protocol number and Enter to print a particular protocol.

2.25.3 Creating and editing protocols

To create a new protocol with default values, press E (Edit). Choose a protocol number not in the list and press Enter. The first free protocol number is shown in the menu. Protocols have already some values in them, either default or old parameters. If you want to set default parameters, press F (deFault) and select the protocol number.

You will be asked for a line number when starting editing a protocol. Press Enter to start editing from the beginning of the protocol or type a line number followed by Enter to edit a certain protocol line. You can always jump between protocol lines by pressing L followed by a line number and Enter. Type P and press Enter to jump to the previous line. The up and down arrow keys can also be used.

The value in front of the arrow on each line is the default value. Just press Enter if you do not want to change this value, or type a new value and Enter if it must be changed. The Ctrl-B (Ctrl key held down and the B key pressed) and Ctrl-N keys can be used to move within the input text; the left and right arrow keys can also be used. See 2.1.4 Editing input data.

NOTE: Unnecessary lines in a protocol can be hidden to make the editor shorter. Press Ctrl-K (Ctrl and K keys pressed at the same time) to hide the current line. To reveal a hidden line, go to the next line and press Ctrl-U. To unhide all hidden lines go to line selection line in the beginning of the editor and press Ctrl-A.

Press / and Enter when you are finished with your editing and want to save the changes made. If you want to exit without saving the new values, press \ and Enter.

2.25.4 Example protocol listing

The example alongside shows a typical dual label protocol listing. The parameters are described in section 2.25.7.

```
Counting protocol no: 26 Mon 24-May-2000 10:49
Name: Dual CPM
CPM normalization protocol no:
Wallac 1450 MicroBeta V4.4 S/N 4500759
 1 Protocol name:                          Dual CPM
 2 Counting mode:   1)CPM  2)DPM                1
 3 CPM normalization protocol                   -
 4 Number of labels:  1)Single  2)Dual          2
 5 Isotope 1:  1)H-3     2)I-125      3)C-14
               4)S-35    5)Cr-51      6)P-32
               7)P-32 Cerenkov        8)Other   1
     ParaLux (Y/N)                              N
     PMT use:  1)Normal    2)Upper     3)Lower  1
     Window 1:                              5-360
 6 Isotope 2:  1)H-3       2)I-125     3)C-14
               4)S-35      5)Cr-51     6)P-32
               7)P-32 Cerenkov         8)Other  3
     Window 2:                             150-650
10 Counting time [s]                            60
12 Precision (2 sigma) [%]                     0.2
20 Count all positions in cassette (Y/N)         N
21 Cassette type:  1)96  2)24                    1
22      Cassette 1:(96)     Active rows:       AB
                              Row A: 111111111111
                              Row B: 111100000000
                              Row C: 000000000000
                              Row D: 000000000000
                              Row E: 000000000000
                              Row F: 000000000000
                              Row G: 000000000000
                              Row H: 000000000000
         Cassette 2:(96)    Active rows:     ACEG
23 Plate orientation:  1)Normal   2)Rotated
                       3)Mirrored 4)Both         1
30 Printer output:  1)No     2)Short
                    3)Long   4)Programmable      4
31 Printer output:POS CTIME SQP(I) CCPM1 CCPM1%
                  :CCPM2 CCPM2% PROT SPECTRA
      Plot energy scale: 1)Logarithmic [ch]
                         2)Linear [keV]
                         3)Bothspectra           1
      Plot area [channels]                  1-1024
      Plot size:  1)Small  2)Medium  3)Large     2
32 Display output: 1)No      2)Short
                   3)Long    4)Programmable      3
34 External output:1)No      2)Short
                   3)Long    4)Programmable      1
36 Fileoutput:     1)No      2)Short
```

```
                  3)Long    4)Programmable     4

37 File output: POS CTIME SQP(I) SQP(I)% COUNTS1
              :CCPM1 CCPM1% COUNTS2 CCPM2
              :CCPM2% FLAG STM PROT
   File path:                      C:\MB1450\TEST\
   File name extension                          1
40 Change special features (Y/N)                Y
41 Counting control (Y/N)                       Y
     Number of repeats                          3
     Number of replicates                       1
     Number of cycles                           1
42 Background correction (Y/N)                  Y
     Use normalization background (Y/N)         N
     Background sample (Y/N)                    Y
     Bgnd sample position                     G12
     Bgnd counting time [s]                    60
43 Half-life correction (Y/N)                   Y
     Half-life 1 [h]                   107500.00
     Zero time 1 set (Y/N)                      Y
     Zero date 1                      5-Mar-2000
     Zero time 1                        10:18:46
     Half-life 2 [h]                        0.00
44 Chemiluminescence correction (Y/N)           N
45 Use password (Y/N)                           N
48 Delay between plates [m]                     0
```

2.25.5 Protocol copy and delete

The contents of one protocol can be copied to another by pressing C (Copy). Select the No. of the protocol to be copied and enter the new protocol number. If you want to delete a protocol, press D (Delete) and select the protocol number. Press Q (Quit) to return to the Ready state.

2.25.6 Protecting protocols

Protocols can be protected against unauthorized editing by using passwords. The password is selected on line 45 in the protocol editor. This prevents other users from changing or deleting your protocol, but they can still use it (e.g. listing, copying and counting with it). The MicroBeta supervisor has the authority to list the passwords.

2.25.7 Parameters in a counting protocol

1. Protocol name

Type a name for the protocol, maximum 170 characters (only the first 16 characters will be shown in the protocol list) and press Enter. The name may not start with characters ?, / or \.

2. Counting mode

Type 1 for CPM counting or 2 for DPM counting.

3. CPM normalization protocol / DPM standardization protocol

Type the number for the normalization (in CPM counting) or standardization protocol (in DPM counting) used. Type H or ? to get a list of normalization/standardization protocols. Isotopes must be entered if no protocol is selected, otherwise the counting protocol uses the same isotope(s) as in the norm./std. protocol. Type - if no protocol is needed. This means that no normalization correction is done in CPM counting and no DPMs are calculated in DPM counting. Lines 4 to 5 are displayed only if no normalization or standardization protocol number is selected. Information about whether norm./std. has been done is given after line 3.

4. Number of labels

Type 1 for single label counting (one isotope, default) or 2 for dual label counting (two isotopes).

5. Isotope 1

Select an isotope, in dual labelled samples the isotope with the lower energy. The alternatives are:

1) ^{3}H (default) 2) ^{125}I

3) ^{14}C 4) ^{35}S

5) ^{51}Cr 6) ^{32}P

7) ^{32}P Cerenkov 8) Other

9) Luminescence

You will be asked for window limits if you select 'Other', otherwise window settings for the selected isotope are shown.

ParaLux counting can be selected in single label non-crosstalk and non-luminescence protocols. If ParaLux is selected, then AQP(I) is used instead of SQP(I). ParaLux counting has two modes: High efficiency and Low background. The discriminator channel that is used to calculate AQP(I) and High efficiency counts can also be optimized.

For PMT use select normal coincidence counting, or use only upper or lower photomultiplier tubes. Upper is the default if luminescence counting is selected.

6. Isotope 2

Displayed only in dual label counting. The higher energy isotope is selected. The alternatives are the same as on line 5 with ^{14}C as default isotope.

7. Window 1

Displayed only if 'Other' has been selected as isotope 1. Enter the limits for window 1 in a range from 1 to 1024.

8. Window 2

Displayed only if 'Other' has been selected as isotope 2. Enter the limits for window 2 in a range from 1 to 1024.

9. Window 3

Displayed only if 'Other' has been selected as isotope 1 or isotope 2 in dual label counting. Enter the limits for window 3 in a range from 1 to 1024 or 0 - 0 if no third window is used.

10. Counting time

The counting time is given in seconds. The minimum value is 0.1 and the maximum is 999 999.9 seconds. The default value is 60.0. If quick view is selected then the maximum plate time is requested. This is the total counting time of the plate without the sample change time. The default value is 960.0.

12. Precision (2 sigma)

Counting stops when the standard deviation is below the two sigma value in all detectors. Precision is given in percent in a range from 0 to 99.9. 0 means that no precision limit is used. The default value is 0.2 %.

20. Count all positions in cassette

Press Y (Yes) if you want to count the whole cassette (default) or N (No) if you want to specify the positions to be counted. If No has been selected the next parameter will appear:

21. Cassette type

Type 1 if you use cassettes with 96 wells (default) or 2 if the cassettes have 24 or 384 wells.

22. Active rows

If you answered No on line 20 you will have to specify the rows to be counted for the first cassette by typing the letters for these rows. E.g. 'AC' means that samples A1 to A12 and C1 to C12 will be counted. For a more detailed specification, i.e. giving the positions instead of rows only, press R (Row coding). In this case specify which positions are to be counted for each row. The positions are in order from 1 to 12 (or 6 for 24-well plates). Type 1 to count a position or 0 to skip it. E.g. Row A: ->0011110 means that positions A3, A4, A5 and A6 should be counted. You do not have to enter all the zeros at the end of the line; the row end is filled with 0 or 1 according to the last typed character. See the following example:

```
20 Count all positions in cassette (Y/N)        Y ->N
21 Cassette type:  1)96  2)24                   1 ->
22      Cassette 1:(96)     Rows:              AB ->R
                              Row A: 111111111111 ->0011110
                              Row B: 001111000000 ->0000110
                              Row C: 000011000000 ->0
                              Row D: 000000000000
        Cassette 2:(96)     Rows:         ABCDEFGH ->AB
        Cassette 3:(96)     Rows:         ABCDEFGH ->/
```

An alternative method is to type the column numbers e.g. 5,6,7 equals 000011100000, 1, equals 100000000000 and 3,, equals 001111111111.

After pressing Enter you will be asked for positions for the next row. The default values are the same as you have given on the previous row.

Press / when you want to stop specifying counting positions. The rest of the rows will then be counted as the last edited row. In the example above, the following positions will be counted for cassette 1: A3, A4, A5, A6, B5 and B6. The following cassettes can then be coded in the same way as the first one. Press / once again to exit cassette coding. If there are more cassettes in a batch than there are defined cassettes, the rest of them are counted according to the last cassette specification.

W can be pressed to code additional cassettes later.

X can be pressed to change the cassette type (96 to 24 (or 96 to 384) and vice versa) for cassettes 2 to 16, see the example:

23 Plate orientation

Select 2 if rotated plates (A1 at the bottom right corner) are used, 3 if mirrored (A1 at the top right corner), and 4 if both rotated and mirrored (A1 at the bottom left corner).

30. Printer output

There are four printer output alternatives:

1) No
2) Short
3) Long (default)
4) Programmable

1) No
If No is selected no results are printed.

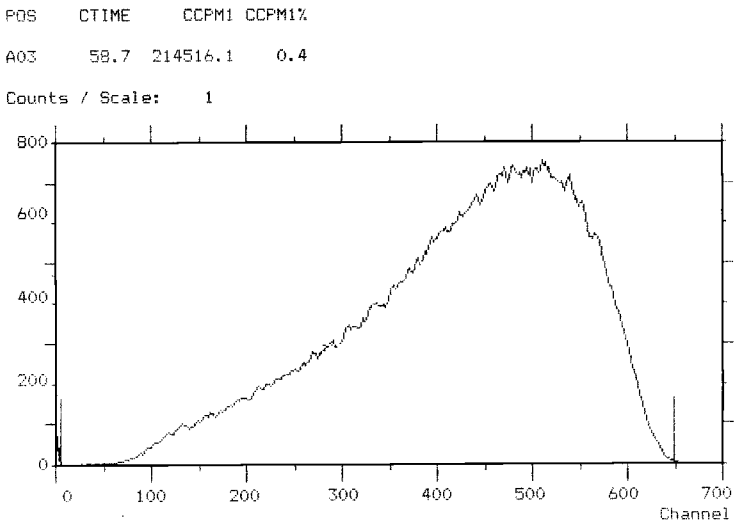
2) Short
If Short is selected, sample results are printed using the short results format: `POS, CCPM1, CCPM1%`. In dual label `CCPM2` and `CCPM2%` are added. `CCPM` is replaced with `DPM` in DPM counting. In luminescence counting it is `POS, LCPS` and `LCPS%`.

3) Long
The long output contains: `PROT, POS, CTIME, SQP(I)` (or `AQP(I)`), `CCPM1, CCPM1%`, plus CCPM2, CCPM2% in dual label counting. In DPM counting `DPM1` and `DPM1%` are added. In luminescence counting the output is `PROT, STIME, POS, CTIME, CPS1, LCPS, LCPS%`.

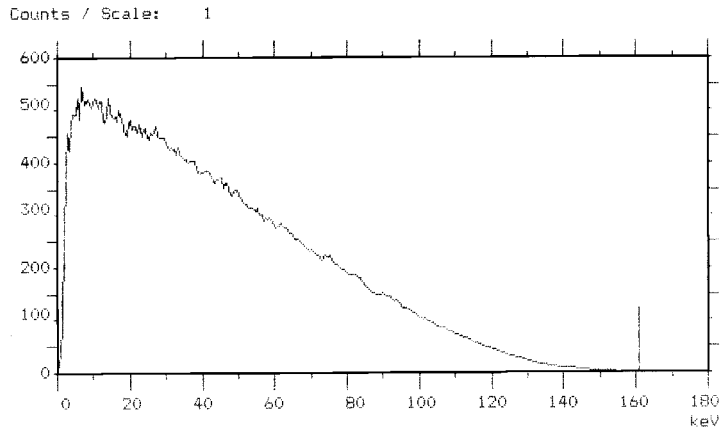
4) Programmable
The output items and arithmetic expressions are defined separately on the next line. Sample quality monitors can also be selected in the programmable output. Press ? or H to get a list of output items, monitors and rules for using own formulae. See also chapters 2.1 'Beginning operation', 2.26 'Results', 2.29 'Statistics' and 2.21 'Monitors and flags'.

If SPECTRA is selected, three more lines are shown:

The first asks for the energy scale to be selected. It can be logarithmic with the units in channels or linear with the units in keV or both of these. Next you are asked to specify the range of the plot. This will be channels or keV depending on your previous selection. Finally you are asked for the spectrum size, small, medium or large. A smaller size will give faster output. The two figures following illustrate the type of plots you may get.



Spectrum with logarithmic scale



Spectrum with linear scale

32. Display output

This output is selected when you want to display results on the screen. It can be chosen independently of the printer output.

Display output has the same output alternatives as the printer output.

The default programmable output, `PRINTER` means the same data as specified for printer output.

34. External output

This output is selected when sending data to an external device such as a mainframe computer connected to the MicroBeta via port 2. The data is in ASCII format (text files).

The output alternatives are the same as in printer and display output, except that alternative 1, No output, is the default value.

The default programmable output, PRINTER means that the same data is specified as for printer output.

36. File output

The counting results can be stored on a floppy disk or hard disk on the PC or MicroBeta, or on the server of a local area network (LAN) (section 2.8 Datafiles). The default setting is 1, no file output. The alternatives are the same as in printer output.

The default programmable output, PRINTER means that the same data is specified as for printer output.

The result files are named so that the first part of the name (the part before the period) is the protocol number and the second part (after the period) is a running number indicating the assay's execution order. E.g. the first file for protocol 5 is named 5.001, the second 5.002, etc.

Result file names for normalizations begin with N (e.g. N1.001) and standardizations with an S (e.g. S1.001).

The disk drive (A:, B: for floppy disks, C: and D: for hard disks, F: etc. for LAN drives) and the path (e.g. \MB\DATA\) for the result files are chosen after the File output. This over-rides any path set in System mode. The drive and path defined in the System state is the default value (see chapter 2.30.9 System/Data drive).

This is the terminal PC path. If results should be stored on the MicroBeta disk, then the path must begin with an @, e.g. `@B:\DATA\`.

If a VT52 or VT100 terminal is used then results are stored on the protocol disk as default (directory `B:\RESULTS`), see 2.30.10 Terminal.

The file name extension (the part after the period) can be changed on the next line. This must be a number in the range 1 to 999.

Note: this line is not available if UltroTerm is used.

40. Change special features

Press Y to change special features. These are counting control, background, half-life and chemiluminescence correction, password and special plate type.

41. Counting control

This and the following questions will be displayed only if Yes has been selected on the previous line.

Press Y to change the counting control parameters. These are number of repeats, replicates and cycles.

If N is selected then these parameters have no effect.

The number of repeats is the number of times each sample will be counted. The minimum value is 1 (default) and the maximum is 99. If repeat is greater than 5, results are not sorted and statistical results are not printed. Repeats cannot be selected in crosstalk.

The number of replicates specifies how many replicates there are for a sample. This is used for evaluating sample preparation errors. The minimum value is 1 (default) and the maximum is 99.

The number of cycles is the number of times a batch of sample cassettes will be counted. The minimum is 1 (default) and the maximum is 99. If the number of cycles is greater that 1 then you will be asked to give the Cycle delay. This is in minutes and is the time between when one cycle ends and the next one begins.

See chapter 2.6 Counting control for more information.

42. Background correction

Press Y to use background correction. You can choose between using the background measured in normalization, giving the position for a background sample or giving a value to be subtracted from the sample CPM. Answer Yes to the background sample question if you want to use a background sample. Enter the position for this sample. H12 (96-well, 1-6 detectors), G12 (12-detectors), D6 (24-well) or P24 (384-well, 1-6 detectors) is the default. Enter also the counting time (60s is the default). Note that the background sample must be in a position reached by every detector (see chapter 2.9 Detectors for these positions). The measured backgrounds are subtracted from CPMs when calculating CCPMs.

Answer No to the background sample question if you want to give a background value. The value, given in CPM, is subtracted from the sample CPM for an isotope. Two values are given,

one for each window, if dual label counting is used. In dual label DPM counting, three values are given. The value is in the range from 0.0 (default) to 99.9 CPM or 999 999.9 LCPS.

Note: in the normalization protocol press Y if you want to count the background plate.

43. Half-life correction

Press Y if you want to use half-life correction. The half-life for the isotope is displayed, e.g. 107 500 hours for ^{3}H. No half-life value is given for ^{14}C because it is so long. Note that you must always specify the half-life if you have selected an isotope of type 'Other'. It must be in a range from 0 (no half-life) to 999 999.99 hours (114 years).

If normalization or standardization protocol is selected on line 3 then half-life is as specified in this protocol and it cannot be changed in the counting protocol

Press Y on the next line if you want to set zero time, otherwise the activity is corrected to the time when counting of the first sample of the assay was started.

The zero time consists of date and time. First enter the date in the format `DD-MMM-YYYY`, where `DD` is the day number (1 - 31), `MMM` the month in letters (`JAN, FEB, MAR, APR, MAY, JUN, JUL, AUG, SEP, OCT, NOV` and `DEC`) and `YYYY` the year, e.g. `15-JUN-2000`. The date can also be in the format `YYYY-MM-DD`, e.g. `2000-06-15`. Then enter clock time in the format `HH:MM:SS`, where `HH` is hours in the 24 hour mode (0 - 23), `MM` minutes (0 - 59) and `SS` seconds (0 - 59), e.g. `14:05:30`. It is not necessary to give seconds. The default value for zero time is the current time. See also chapter 2.14 Half-life.

44. Chemiluminescence correction

Press Y to use chemiluminescence correction, otherwise N (default). This correction is done with a correction formula, see chapter 3.3 Calculation methods.

45. Password

Press Y to select a password if you want to prevent other users from changing your protocol. Enter the password, maximum 12 characters. This will NOT be echoed to the screen. You will also be asked to retype it to prevent typing errors. The protocol cannot later be edited or deleted without knowing the password. You must therefore try to remember it. Only the MicroBeta supervisor has the authority to list the passwords (see chapter 2.30 System/Passwords). He or she can help you if you have forgotten your password.

46. Special plate

Select Special plate type. Wallac is the standard plate. Betaplate filtermat plates and other 24-well plates have different dimensions and are counted using just one detector.

However, it is possible to count other 24-well plates with all detectors. This selection comes after the plate dimension questions. For Betaplate filtermats you can select the 96-well plate format output. This is a composite of four 24-well plate format outputs. If you select the Other option for plate type, you must give the plate dimensions. These are the distances of the centres of the A1, A6 and D1 wells from the plate sides. Special cassettes must be used for Betaplate filtermat plates and other 24-well plates. The default dimensions 17.3/ 13.4/113.6/71.5 are for the Costar plate type. For the Falcon plate type the dimensions 14.5/13.4/110.7/71.5 should be used.

The default dimensions for a special 384-well plate are 11.8/9.0/115.3/76.5. These are the distances of the centres of the A1, A24 and P1 wells from the plate sides.

Note: this line is not available if 96-well cassette has been selected elsewhere in the protocol.

47. Autoquench correction

Automatic quench correction can be done in single label CPM counting. Isotopes must be 1, 2, 3, 4 or 6, and a non-crosstalk CPM normalization protocol must be selected.

48 Delay between plates

The delay between the end of plate measurement and the start of the next plate measurement can be specified. The range is 0 to 9999 minutes.

2.25.8 Parameters in a normalization protocol

The parameters are much the same as in the counting protocol with some exceptions. Information whether normalization/standardization has been done is given after the protocol name. The following counting protocol parameters are not included in the normalization protocol:

```
2.  Counting mode
3.  CPM normalization/DPM standardization protocol
20. Count all positions in cassette
21. Cassette type
41  Counting control
```

The following parameters are new or changed when compared with the ones in the counting protocol:

3. Crosstalk correction

Press Y to use crosstalk correction otherwise select N. see chapter 2.7 Crosstalk correction for further information.

10. Counting time for background

The counting time for the background and crosstalk counting samples in crosstalk counting is given in seconds. The minimum value is 0.1 and the maximum is 999 999.9 seconds. The default value is 60.0.

11. Counting time for standards

The counting time for the standard samples is also given in seconds, from 1 to 999 999.9 with 60.0s as the default value.

41. Isotope activity setting

Press Y to set activities for normalization samples. This is isotope dependent and given in DPM. The range is from 1000.0 to 999 999.9. The default value is 200 000 for isotope 1 and 100 000 for isotope 2.

If activities are given, absolute normalization is done with efficiencies as count rates divided by the given activity.

If N is selected, relative normalization is done with efficiencies as a fraction of the highest detector count rate.

47. Strip plate

Press Y if you use plates where crosstalk is different in the X and Y directions. The crosstalk sample in G10 is counted in this case.

See chapter 2.23 Normalization for further information.

2.25.9 Parameters in a standardization protocol

The parameters are much the same as in the counting and normalization protocols with some exceptions. Information whether normalization/standardization has been done is given after the protocol name. The following counting protocol parameters are not included in the standardization protocol:

```
2.  Counting mode
3.  CPM normalization/DPM standardization protocol
20. Count all positions in cassette
21. Cassette type
41. Counting control
42. Background correction
```

The following parameters are new or changed when compared with the counting protocol:

2. Sample type

Select normal or scintillation proximity assay for sample type. ParaLux counting is set automatically if SPA is selected.

3. Crosstalk correction

Press Y to use crosstalk correction otherwise select N. See chapter 2.7 Crosstalk correction for further information.

14. Easy DPM

Press Y to use Easy DPM quench curves. Only the two DPM normalization samples are counted in standardization.

20. Number of standards

Give the number of DPM standards used for standardization. The value must be in a range from 2 to 12 (6 in dual label) with 6 as default.

21. Isotope 1 activity

Give the isotope activity in DPM. The range is from 1000.0 to 999 999.9 with 200 000 as the default value.

23. Isotope 2 activity

Give the Isotope 2 activity in DPM. The range is from 1000.0 to 999 999.9 with 200 000 as the default value.

24. Standard curve fit selection

Press Y to use standard curve fitting, otherwise N. If No is selected, smoothing spline with automatic smoothing will be used or linear interpolation if SPA is selected for the sample type.

25. Curve fit method

This line is displayed only if you have selected Yes on the previous line. The methods for standard curve fitting are:

1) Smoothing spline (default)
2) Interpolation spline
3) Linear interpolation (default for SPA)
4) Linear regression

Select a method by typing the number and Enter. If you have selected the first alternative, smoothing spline, you will be asked if the method is to include automatic smoothing. Press Y

(default) if so. Otherwise press N and you will be asked for a smoothing parameter. Enter a value between 0.0 (no smoothing) and 1000.0 (maximum smoothing), with 1.0 as default.

26. Edit standard curve

Press Y to edit the quench parameter, efficiency and weight values for a standard curve or to delete curve points.

41. Adjusted activities

Press Y if you want to adjust the activities for standards, otherwise the activity given on line 21 will be used for every standard. After pressing Y you will be able to change activities for the same number of DPM standards as given on line 20. You can also change the activities for the two Easy DPM standards. The range is from 1000.0 to 999 999.9 DPM. The value given on line 21 is default. See chapter 2.12 DPM Standardization for further information.

2.26 Results

2.26.1 Output items

Different kind of output items can be entered after selecting Programmable output (format 4) on lines:

```
30 Printer output
32 Display output
34 External output
36 File output
```

The possible output items are shown in the tables following:

Note: in these tables y stands for 1 or 2 or 3 and z for 1 or 2

(a) Sample identification data:

```
AS      = Value of assay ID for the current plate (0-99)
POS     = Sample position on microtitration plate (A01-H12/P24)
SEQ     = Running sample number (1=A01,96=H12,97=A01 on 2nd plate etc.)
PR      = Protocol number used (0-99)
CA      = Value of cassette ID for the current plate (0-9999)
FU      = Value of function code for the current plate (NORM, STD, STOP)
RP      = Repeat number of the current measurement
D       = Detector number (1-6)
S       = Spectrum half (A,B).
CT      = Cassette type (24, 96, 384)
SH      = Shelf number (1-16/32)
CNO     = Cassette order number (1,2,3,...)
PLATEID = Plate ID
IM      = Injector module
IMI     = Injector module index
```

(b) Sample counting data:

```
CTIME   = Dead time corrected counting time in seconds
ETIMEz  = Elapsed time in hours for isotope z from zero time z
STIME   = Counting start time (HH:MM:SS)
DATE    = Current date (DD-MMM-YYYY)
TIME    = Current time (HH:MM:SS)
SQP(I)  = Spectrum quench parameter (0.0-1024.0)
SQP(I)% = Percentage theoretical error of spectrum quench parameter
ISEP    = Isotope spectrum end point (0.0-1024.0)
ISEP%   = Percentage theoretical error of ISEP
COUNTSy = Counts for isotope z (or extra window 3)
CPMy    = Count rate (counts per minute) for isotope z (or extra window 3)
CPMy%   = Percentage theoretical error of CPMy (i)
CCPMy   = Corrected count rate for isotope z (or extra window 3) (ii)
CCPMy%  = Percentage theoretical error of CCPMy (i)
DCCPMy  = Absolute theoretical error of CCPMy (i)
```

```
DPMz    = DPM of isotope z (ii)
DPMz%   = Percentage theoretical error of DPMz (i)
DDPMz   = Absolute theoretical error of DPMz (i)
CLM%    = Chemiluminescence in window 1
EFFy    = Isotope z (or extra window 3) counting efficiency
STM     = Statistics monitor (iii)
DPMM    = DPM out of range monitor (iii)
FLAG    = Termination flag (iii)
Wy      = Window y limits
CPSy    = Count rate (counts per second) for isotope z or extra window 3
CCPSy   = Corr. count rate (counts per sec.) for isotope z
          (or extra window 3)(ii)
RESP1   = CCPM1 or LCPS
RESP2   = CCPM2
RESPz%  = Percentage theoretical error of RESPz
LCPS    = Luminescence corrected counts per second divided by 100
LCPS%   = Percentage theoretical error of LCPS (i)
DLCPS   = Absolute theoretical error for LCPS (i)
LM      = LCPS out of range monitor (iii)
AQP(I)  = Asymmetric quench parameter (0.0 - 1024.0)
AQP(I)% = Percentage theoretical error of asymmetric quench parameter
CPM_C   = Coincidence CPM1
CPM_NC  = Non-coincidence High efficiency CPM1 = CPM_C + CPM_NC
CPM_AQP = CPM used in AQP(I) calculation
```

(c) Statistical data:

```
STAT    = The data specified after this will be output only when a
          repeat or replicate mean value is available
S1      = Selects 1 sigma level for all deviation output fields (i)
REPL    = Replicate sample range (e.g. A01-A03 if A01, A02 and A03 are
          replicates)
MEANz   = Mean value of CCPMz, DPMz or LCPS
CVz%    = Percentage coefficient of variation for CCPMz, DPMz or LCPS (i)
SEMz%   = Percentage standard error of mean for CCPMz, DPMz or LCPS (i)
TCVz%   = Percentage theoretical coeff. of variation for
          CCPMz, DPMz or LCPS (i)
TSEMz%  = Percentage theoretical stand. error of mean for
          CCPMz, DPMz or LCPS (i)
SDz     = Standard deviation for CCPMz, DPMz or LCPS (i)
SEMz    = Standard error of mean for CCPMz, DPMz or LCPS (i)
TSDz    = Theoretical standard deviation for CCPMz, DPMz or LCPS (i)
TSEMz   = Theoretical standard error of mean for CCPMz, DPMz or LCPS (i)
CHISQz  = Reduced chi-square value for CCPMz, DPMz or LCPS
Pz      = Probability as a percentage for CCPMz, DPMz or LCPS
```

(d) Controlling selections:

```
SPECTRA = Plot spectra (Printer output only)
NEWLINE = Start new line
NL      = Start new line (shorthand)
SPSAVE  = Output spectra in compressed numerical format
PROT    = Include protocol listing in the beginning of output
```

```
PLATE   = Output results in microtitration plate format (see the chapter
          Microtitration plate format output)
PRINTER = Output same data as specified for printer (iv)
TXT     = Produces "Start of assay:nn"-message
TAB     = Produces tab-character (ASCII 9) between all output columns to
          ease loading of the result file into commercial programs
          e.g. spreadsheets
EXCL    = Controls excluding of unrequired parts of the output (v)
FF      = Sends a form feed character to the printer after the
          "End of assay" message
NOLIVE  = No status display
```

Notes:
(i) Statistical deviation values will be output normally on the 2-sigma level. However if item S1 exists in an output selection then for that output device, deviation values will be printed on the 1-sigma level.

(ii) Corrections made:

(a) Background correction if in protocol either:
`42 Background correction = Y (Yes)`
and
`Use normalization background = Y (Yes)`
or
```
Background sample     = Y (Yes) or
Background subtraction 1 >0.0 (CCPM1 or DPM1) or
Background subtraction 2 >0.0 (CCPM2 or DPM2).
```

(b) Half-life correction if in protocol:
`43 Half-life correction = Yes`
and
```
Half-life 1 > 0   (CCPM1 or DPM1) or
Half-life 2 > 0   (CCPM2 or DPM2).
```

(c) Chemiluminescence correction if in protocol:
`44 Chemiluminescence correction = Yes`

(d) Detector efficiency and isotope spillover correction if normalization has been done for the protocol

e) Quench correction if standardization has been done

f) Crosstalk correction if in normalization or standardization
`3 Crosstalk correction = Yes`

(iii) Monitors and flags in output (see chapter 2.21 Monitors and flags)

a) Statistics monitor checks count rate variations in a sample and gives the values:

```
.       No statistic variation detected
REC1    No variation after one recount
REC2    No variation after two recounts
ERR     Still variation after two recounts
```

b) DPM out-of-range monitor, selectable in DPM counting only, checks quench levels in samples by comparing with the quench in standardization. The following values can be displayed:

```
.       OK, no out of range detected
OUT     DPM out of range
```

c) LCPS out-of-range monitor, selectable in luminescence counting only, checks counter saturation. The following values can be displayed:

```
.       OK no out of range detected
HIGH    counter saturation detected
?       luminescence scaler is used (saturation cannot be detected)
```

d) Termination flag tells if counting has been stopped. The values for FLAG can be:

```
.       Normal termination
PREC    Precision reached
MAX     Hot sample (more than 50 000 counts/channel)
INT     Interrupted by user
```

(iv) Applicable on lines 32 (Display output), 34 (External output) and 36 (File output). Selects same output as was selected on line 30 (Printer output). However PRINTER does not select automatically protocol listing (PROT) although PROT exists in printer selection. E.g. to select protocol listing for file output you must specify PRINTER PROT.

(v) The EXCL output item allows you to customize your output. This can be useful if, for example, you want to prepare output for a spreadsheet program.

The normal output format of an assay is shown below in the table in the next column.

```
32-> Data file: F:\USR\GUEST\IHME\33.003

16-> Counting protocol no:33 Thu 27-May-2000 15:38
16-> Name: TEST
16-> CPM normalization protocol no: 40

8->Assay:-/ Prot:33/ Cass:-/ Func:-/
   Cassette no: 1/  Shelf:3     /4*6
   PlateID:

4-> POS   CTIME   CCPM1   CCPM1%

    A01   29.7    90680   1.2
   ...
    A06   29.7    90972   1.2

1->

    B01   29.7    86029   1.2
    ...
    B06   29.7    75349   1.3

    ...
    D06    29.7   75025   1.3

2->

8->Assay:-/ Prot:-/ Cass:-/ Func:-/

   Cassette no. 2/  Shelf:4      /4*6

4->POS   CTIME   CCPM1   CCPM1%

   A01   29.7    90652   1.2
   ...
   A06   29.7    90963   1.2

1->

   B01   29.7    86122   1.2
   ...
   B06   29.7    76239   1.3
   ...

   D06   29.7    75198   1.3

16-> Total count rate:4130593.2 CCPM
                      1859.56 nCi68.83 kBq

32-> End of assay
```

You will notice that certain lines are tagged with a number and an arrow. In normal operation these line tags are hidden but they are shown here to help explain this function.

The EXCL-keyword can be used to mask out of the output whichever of the tagged lines you want left out. To do this you must use the code EXCL followed by two numbers separated by colons ":". The format is

```
EXCL:<begin>:<rest>
```

where:

<begin> sum of the numbers used to tag lines you want to exclude before and during the first cassette output (add 64 if not greater than <rest>, see below)

<rest> sum of the numbers used to tag lines you want to exclude during output from other cassettes and after the last cassette.

The numbers which can be used (as the example shows) are for <begin>:

32 - datafile path
16 - Protocol name, number and type
8 - ID label contents and shelf number
4 - output column headings
2 - empty line after results from one cassette
1 - empty line after results from one row

For <rest> the numbers are:

32 - end of assay
16 - total count rate
8 - ID label contents and shelf number
4 - output column headings
2 - empty line after results from one cassette
1 - empty line after results from one row

A number resulting from the addition of any combination of these numbers can only be formed by that combination. So when you give the sum to the counter it knows what combination of these numbers has gone into making it and hence which lines to omit from the output.

Example: `EXCL:72:12` excludes the ID-line before the first cassette (<begin>=64+8) and excludes the ID-line and headings before the other cassettes (<rest>=8+4). Note that here 64 was added because 8<12.

2.26.2 Changing the format of an output field

The format of an output field (width of field and number of decimals) can be changed. The syntax is:

```
<heading>:<width>
```

or:
<heading>:<width>:<decimals>
where:
<heading> = One of the output items in groups (a), (b) and (c) listed above (except S1,STAT,REPL).
<width> = The width of the output field in characters (including <decimal>). The program adds an extra space character between printout fields.
<decimals> = Number of digits to the right from the decimal point (works for real number values such as CCPM1 only). In the examples following character # denotes a leading space character and it is supposed that the value of the CCPM1 field is 1234567.123456.

Examples are given in the table at the head of the next column:

FORMAT SPECIFICATION	OUTPUT OBTAINED	EXPLANATION
CCPM1	1234567	Default format (=CCPM1:7:0)
CCPM1:13:3	##1234567.123	13 characters, 3 decimals
CCPM1:13:4	#1234567.1235	Last digit 5 because of rounding
CCPM1:12:4	1234567.1235	Exactly 12 digits in field
CCPM1:11:4	1234567.123	Value too large strip off a decimal
CCPM1:9:4	1234567.1	Value too large strip off 3 decimals
CCPM1:8:4	#1234567	Value too large strip off all decimals
CCPM1:7:4	1234567	Value fits exactly without decimals
CCPM1:6:4	1.2E006	Value too large show value in scientific notation. 1.2E006 means 1.2 times ten to the power 6.

2.26.3 Entering constant text into an output

Text can be defined between apostrophes (). The syntax is:
<text> or <text>:<width>
where:
<text> = Arbitrary commentary text.
<width> = Width of the text field. If this is greater than the number of characters in <text> then leading spaces will be produced.

Examples:

SPECIFICATION	OUTPUT	EXPLANATION
"Example"	Example	Character inside ""s shown.
"Example":5	Example	Format has no effect (format is less than length of Example).
"Example":10	###Example	3 leading spaces output resulting in a total of 10 characters in a field.
""		Empty text. No output.
"":10	##########	A method to produce 10 spaces.

2.26.4 Defining new output fields

New output fields can be defined. The syntax of a new output is:
[<heading>=<expression>]
or
[<heading>=<expression>]:<width>
or
[<heading>=<expression>]:<width>:<decimals>
where:
<heading> = The heading for the new output field. This must not be included in the headings listed in paragraph (2.26.1) above. It consists of letters and digits starting with a letter.
<expression> = An arithmetic expression consisting of fixed headings listed in paragraph (2.26.1)(a) and (b) above (excluding POS, S, STM, DPMM, LM, FLAG, W1, W2 and W3), numerical constants and operators listed below. Note that statistical values cannot be used in expressions.
<width> = The width of a printout field in characters (default 12).
<decimals> = Number of digits to the right from the decimal point (default 4).

Operators allowed are:

+	Addition	e.g. CCPM1+100
-	Subtraction	e.g. CCPM1-100
*	Multiplication	e.g. 100*CCPM1
/	Division	e.g. CCPM1/100
^	Powerfunction	e.g. CCPM1^0.5 = Sqrt(CCPM1)
Abs	Absolute value	e.g. Abs(CCPM1-100)
Ln	Natural logarithm	e.g. Ln(CCPM1)
Exp	Exponent function	e.g. Ln(Exp(CCPM1)= CCPM1
Sqrt	Square root	e.g. Sqrt(4.0)=2.0)
()	Parenthesis	e.g. 10*(CCPM1+100)

Examples:

SPECIFICATION	EXPLANATION
[CPS1=CPM1/60]	Calculates Counts per second and outputs it in format 9999999.9999.
[RESP=Ln(Abs(CCPM1-102.1087))+0.78]:8:2	An example of a more complicated formula.The output is in the format 99999.99.

Note: If a new output field with the heading AQP is given in any programmable output then this output is used as the quench parameter instead of SQP(I) or AQP(I).

2.26.5 Short output mode

If you select option 2 (short output mode) on protocol lines 30, 32, 34 or 36 then the program produces as output:

```
POS CCPM1 CCPM1% (CCPM2 CCPM2%)
```

Results for each isotope will have their own line.

The corresponding output for DPM counting is:

```
POS DPM1 DPM1% (DPM2 DPM2%)
```

For luminescence counting the output is:

```
POS LCPS LCPS%
```

2.26.6 Long output mode

If you select option 3 (long output mode) on protocol lines 30, 32, 34 or 36 then the program produces as output:

```
PROT POS CTIME SQP(I)(or AQP(I))CPM1 CCPM1 CCPM1% (CCPM2, CCPM2%)
```

Results for each isotope will have their own line.

The corresponding output in DPM counting is:

```
PROT POS CTIME SQP(I) (or AQP(I)) CPM1 CCPM1 CCPM1% (CCPM2 CCPM2%) DPM1
DPM1% (DPM2 DPM2%)
```

For luminescence counting the output is:

```
PROT STIME POS CTIME CPS1 LCPS LCPS%
```

2.26.7 Default formats of output fields

The default format of printout fields is shown in the list here:

Note: Fields are always separated by one space that is not included in the field length.

Note: in these tables y stands for 1 or 2 or 3 and z for 1 or 2

(a) Sample identification data

```
AS:2          xx
CA:4          xxxx
CNO:3         xxx
CT:3          xxx
D:2           xx
FU:4          xxxx
PLATEID:12    xxxxxxxxxxxx
POS:3         xxx
PR:2          xx
RP:2          xx
S:1           x
SEQ:4         xxxx
SH:2          xx
```

(b) Sample counting data

```
AQP(I):6:1    xxxx.x
AQP(I)%:7:1   xxxxx.x
CCPMy:7:0     xxxxxxx
CCPMy%:6:1    xxxx.x
CCPSy:9:1     xxxxxxx.x
CLM%:6:1      xxxx.x
COUNTSy:7     xxxxxxx
CPMy:7:0      xxxxxxx
CPMy%:6:1     xxxx.x
CPM_AQP:7:0   xxxxxxx
CPM_C:7:0     xxxxxxx
CPM_NC:7:0    xxxxxxx
CPSy:9:1      xxxxxxx.x
CTIME:8:1     xxxxxx.x
DATE:11       xx-xxx-xxxx
DCCPMy:9:1    xxxxxxx.x
DDPMz:9:1     xxxxxxx.x
DLCPS:9:1     xxxxxxx.x
DPMz:7:0      xxxxxxx
DPMz%:6:1     xxxx.x
DPMM:4        xxxx
EFFy:6:4      x.xxxx
ETIMEz:9:3    xxxxx.xxx
FLAG:4        xxxx
ISEP:6:1      xxxx.x
ISEP%:5:1     xxx.x
```

```
LCPS:9:1      xxxxxxx.x
LCPS%:6:1     xxxx.x
LM:4          xxxx
RESPz:9:1     xxxxxxx.x
RESPz%:6:1    xxxx.x
SQP(I):6:1    xxxx.x
SQP(I)%:7:1   xxxxx.x
STIME:8       xx:xx:xx
STM:4         xxxx
TIME:8        xx:xx:xx
Wy:13:1       xxxx.x-xxxx.x
```

(c) Statistical data:

```
CHISQz:6:1    xxxx.x
CVz%:6:1      xxxx.x
MEANz:9:1     xxxxxxx.x
Pz:6:1        xxxx.x
REPL:7        xxx-xxx
SDz:8:1       xxxxxx.x
SEMz:8:1      xxxxxx.x
SEMz%:6:1     xxxx.x
TCVz%:6:1     xxxx.x
TSDz:8:1      xxxxxx.x
TSEMz%:6:1    xxxx.x
TSEMz:8:1     xxxxxx.x
```

2.27 Robotic loading interface

(See chapter 2J.27 for JET information)

The robotic loading interface is a special door/adapter system that brings a cassette outside so that a robotic arm can change the plate.

In the MicroBeta program the robotic loading interface is selected as follows:

```
(S)ystem
  (L)evel
    (T)esting
      Detector/shelf (u)sage
        Use shelves: 1)1 2)16 3)32 ->1
  (L)evel
(N)ormal counting.
```

The operation is controlled using the RS-232C port 2 (external port) of MicroBeta. When the cassette is outside, MicroBeta sends the text Ready and carriage return / line feed. The possible commands are:

H or ? - list commands,
Nxx - start normalization using protocol number xx,
Dxx - start standardization using prot. number xx,
xx - start counting using protocol number xx,
A - start counting using IDs.

All Ready commands must be followed by a carriage return / line feed or #. Counting can be interrupted with O. After a plate has been measured and the cassette is outside, MicroBeta sends the text Continue and carriage return / line feed. At this point O stops counting and any other character continues counting.

If plates are counted in the inverted position (A1 at the bottom right corner) then Plate orientation: Rotated should be selected in the counting protocol. This selection reorders results so that outputs POS and SEQ are correct.

2.28 Safety and radioactive materials

The following comments about precautions and safety measures in handling radioactive materials are included as a guide and are not intended to be fully comprehensive. More complete details may be found elsewhere, for example in the booklet SAFE HANDLING OF RADIONUCLIDES, published by the International Atomic Energy Agency, Vienna; this may be recommended as a useful code of practice appropriate to radio-chemical laboratories.

Unless a specially designed radio-isotope laboratory is used, limitations should be placed on the amount of active material in the laboratory area depending on toxicity and type of chemical operation. For high toxicity material and wet chemical operations involving the risk of spillage, the IAEA recommend a maximum activity of about 10 μCi.

Personnel should be properly trained in the safe handling of these materials, maximum levels of stored activities should be set, proper records should be kept, and a definite monitoring schedule maintained.

The areas where samples are handled should be kept clean and free of dust. This is most easily accomplished if all surfaces are as smooth as possible and if the minimum number of extra surfaces is introduced into the room. Lastly it is extremely important to store all radioactive materials in a separate room to which access is restricted.

2.29 Statistics

2.29.1 When statistical output is available

MicroBeta software can produce statistical output values in one of the following situations:

(a) Value of Repeats-parameter (protocol line 41a) is greater than 1 and less than or equal to 5. If Repeats is greater than 5 or if 384-well plates are used then statistical output is not produced.

(b) Value of Replicates-parameter (protocol line 41b) is greater than 1.

(c) Both (a) and (b) hold.

If the user does not specify statistical output values and format then default statistical output format will be used. In this format a table will be output after the last repeat or after the replicate group. The columns of the table are shown below (the symbol used in programmable output is shown in parenthesis):

```
In this table z stands for 1 or 2

MEAN          = Mean value of CCPMz,DPMz or LCPS (MEANz)
THEOR.ERROR(%)= Theoretical standard error of mean as percentages (TSEMz%)
OBS.ERROR(%)  = Standard error of mean as a percentage (SEMz%)
CHI-SQUARED   = Reduced Chi-squared for mean (CHISQz).
PROBABILITY(%)= Probability as a percentage (Pz).
```

See the chapter 3.3 Calculation methods for exact definitions.

The first row of the statistics table shows values for CCPM1, DPM1 or LCPS and the second row (only in the case of a double label assay) shows values for CCPM2 or DPM2. See the printer output of the example in 2.29.3. The default for the above values will be output at the 2-sigma level. If the user uses Programmable output selection and includes symbol S1 in one of the programmable output selections then values will be output at the 1-sigma level.

2.29.2 Restrictions

If the user selects programmable output and includes any of the following statistical values (except S1 and STAT):

```
MEAN1, CV1%, SEM1%, TCV1%, TSEM1%, CHISQ1, P1, SD1, SEM1, TSD1, TSEM1
MEAN2, CV2%, SEM2%, TCV2%, TSEM2%, CHISQ2, P2, SD2, SEM2, TSD2, TSEM2 REPL
```

then a default statistical table summary will not be produced. Instead you must specify in the programmable output selection:

- which statistical values to output,
- how to locate these values in the output and
- which kind of formats are to be used to output the values.

Statistical values cannot be used in arithmetic expressions. The default values will be output at the 2-sigma level unless symbol S1 exists in the selection. The external output of the example in 2.29.3 demonstrates a compact statistical output where only values `MEAN1, SEM1%, MEAN2` and `SEM2%` have been selected. They have been output on the same line as the last replicate in order to reduce paper consumption.

The file output of the example in 2.29.3 demonstrates the use of the control symbol `STAT`. The output values defined after `STAT` will be output only when the last replicate or repeat result has been output. This can be used e.g. to output the statistical data on its own line, as in the example following, or to produce replicate mean values when using Microtitration plate format. See the next page for the examples of statistical output.

2.29.3 Examples of statistical output

The following protocol (only relevant parts shown) demonstrates the different statistical output formats:

```
30 Printer output:   1)No     2)Short
                     3)Long   4)Programmable       3->
32 Display output:   1)No     2)Short
                     3)Long   4)Programmable       3->
34 External output:  1)No     2)Short
                     3)Long   4)Programmable       4->
35 External output: POS CTIME CCPM1 CCPM1% CCPM2 CCPM2% MEAN1 SEM1% MEAN2
                   :SEM2%
   ->
36 File output:      1)No     2)Short
                     3)Long   4)Programmable       4->
37 File output: POS CTIME CCPM1 CCPM1% "":6 CCPM2 CCPM2% STAT NL "MEAN":12
              :MEAN1 TSEM1% SEM1% MEAN2 TSEM2% SEM2%
   ->
     File path:                          C:\MB\TEST\->
     File name extension                           1->
40 Change special features (Y/N)                  Y->
41 Counting control (Y/N)                         Y->
     Number of repeats                            1->
     Number of replicates                         3->
     Number of cycles                             1->
```

Because symbol S1 does not exist in any of the programmable (lines 35 and 37 above) output selections the deviation values are represented at the 2-sigma level. The output defined by the protocol above for each output medium looks like the following:

```
POS    CTIME    SQP(I)      CPM      CCPM     CCPM%  FLAG
A01      9.9     388.3    29737     29737       2.9     .
                         112834    112834       1.5     .
A02      9.9     383.2    29997     29997       2.8     .
                         110761    110761       1.5     .
A03      9.9     375.5    32362     32362       2.7     .
                         111652    111652       1.5     .

3 replicates:

A01-A03     MEAN    THEOR.ERROR(%)    OBS.ERROR(%)  CHI-SQUARED  PROBABILITY(%)
CCPM1   30699.44               1.62            5.44        11.25            0.00
CCPM2  111749.09               0.85            1.07         1.60           20.23
```

2.29.3.1 Printer output

Printer output selection (line 30 above) demonstrates the default statistics output format. In single label counting only a line for `CCPM1` is generated.

2.29.3.2 External output

External output selection (lines 34 and 35 above) demonstrates a very compact statistical output format. The program did not use the default statistical output format because there were statistical output fields in the programmable output i.e. `MEAN1, SEM1%, MEAN2` and `SEM2%`. Here the user was not interested in all of the statistical values which can be selected.

```
POS  CTIME  CCPM1  CCPM1%  CCPM2  CCPM2%    MEAN1  SEM1%     MEAN2 SEM2%
A01    9.9  29737     2.9 112834     1.5
A02    9.9  29997     2.8 110761     1.5
A03    9.9  32362     2.7 111652     1.5  30699.4    5.4  111749.1   1.1
```

2.29.3.3 File output

File output selection (lines 36 and 37 above) demonstrates the use of the control switch `STAT`. The output items to the left of `STAT (POS CTIME CCPM1 CCPM1% :6 CCPM2 CCPM2%)` are output for each individual result. The empty text definition :6 is used to shift `CCPM2` and `CCPM2%` seven (=6+1) characters to the right to leave space for the second line `SEM1%`. The output items to the right of `STAT (NL MEAN:12 MEAN1 TSEM1% SEM1% MEAN2 TSEM2% SEM2%)` are output only when the replicate (or repeat) mean value is available. Then at the first `NL` a new line is output. `MEAN:12` outputs a commentary word `MEAN` under the `CTIME` of the last replicate. `MEAN1 (MEAN2)` outputs a mean value under the CCPM1 (CCPM2) of the last replicate etc.

POS	CTIME	CCPM1 MEAN1	CCPM1% TSEM1%	SEM1%	CCPM2 MEAN2	CCPM2% TSEM2%	SEM2%
A01	9.9	29737	2.9		112834	1.5	
A02	9.9	29997	2.8		110761	1.5	
A03	9.9	32362	2.7		111652	1.5	
	MEAN	30699.4	1.6	5.4	111749.1	0.8	1.1

2.30 System

(See chapter 2J.30 for JET information)

2.30.1 Selecting System

System settings control the general way MicroBeta works - its defaults, hardware connections etc. To enter the System state press S in the Ready state. You are then given a number of options as example 1 shows. These are described in more detail here.

Example 1

```
(H)elp   (I)nfo
(C)ount  (P)rotocols  (S)ystem

Ready>S

(H)elp        (Q)uit            MS-D(O)S
(E)rrorbeep   (S)tatus display  (V)ersion
(C)lock       (R)S-232C         (P)rinter
(D)ata drive  Ter(m)inal        P(a)sswords
Customi(z)ing

System>
```

2.30.2 (H)elp

Press H to get help about the various selections.

2.30.3 (E)rror beep

User or instrument error normally causes a beep. This can be switched off by selecting N.

Example 2

```
System>E

Error beep (Y/N) Y ->N
```

2.30.4 (S)tatus display

Normally when counting is occurring its status is shown on the Terminal PC screen (see chapter 2.5 Counting for more details). The counting status display can be switched off or only currently counted positions can be shown.

Example 3

```
System>S

Status display:  1)No   2)Current positions
                 3)Whole plate                      3->
```

2.30.5 (V)ersion

Show the program and MS-DOS version numbers.

Example 4

```
System>V

MicroBeta program V4.4. Copyright (C)
Wallac Oy, 1997. All rights reserved.

MS-DOS V3.2. Copyright (C) Microsoft
Corporation, 1987. All rights reserved.
```

2.30.6 (C)lock

This allows the date and time to be set. MicroBeta has a calendar clock with battery back-up so you do not have to set it each time the power has been switched off, normally only when the instrument is installed. Details of setting the clock are given in chapter 2.3 Clock. When you set the clock the Terminal PC date and time are also set if GenTerm is used.

Example 5

```
System>C

Date 12-Jul-2000 -> 13-Jul-2000
Time       14:44 -> 9:30

Set clock(Y/N)->Y
```

2.30.7 (R)S-232C

There are three RS-232C communication ports which can be used:
1 for the Terminal PC
2 for an external computer
3 for the printer
You can specify the communication parameters for each of these ports separately. Give the number of the port and press Enter.

The first parameter that can be changed is the speed of transferring the data, the Baud rate. The possibilities are: 300, 600, 1200, 2400, 4800 or 9600.

The default value for the terminal and external ports is 9600. For the printout port it is 4800. If these values are not suitable then select the ones you need.

Next is the parity selection, you can have None, Odd or Even where None is the default.

The number of data bits can be 8 or 7 where 8 is the default.

The number of stop bits can be 2 or 1 where 2 is the default.

Lastly you can select the kind of checking process for the data transfer, the handshaking. It can be None, DTR or XON/XOFF where the default is DTR.

Confirm the selection of these parameters by typing Y twice. When you have done this the program prints the original and the new settings and returns to the System state if serial port 2 or 3 setting is in question. However when changing the terminal port (port 1) setting the program then shows a help text that informs you to set your terminal to the same settings which were selected for MicroBeta before confirming the new setting. When ready press any key. The program should then ask:

```
Do you see this text (Y/N)?
```

If the text appears it is quite sure that the communication works and you can press Y to confirm the change of terminal port setting. If the text does not appear it is obvious that your new terminal and MicroBeta serial port settings differ. In that case check from the printer the new MicroBeta setting and modify your terminal setting if required until you see the above text. If you cannot make the communication work you have still one possibility. Set the terminal to the original serial port setting. Switch off the counter and switch on again. Restarting MicroBeta at this phase returns the original terminal port communication setting. Note: You can lose communication with the counter also by changing the serial port setting of your terminal alone. Usually then some strange characters appear around the screen and commands do not function. To get communication functioning again you can change terminal settings in the following way if you do not remember the terminal port setting of MicroBeta:

- Try different baud rates. Press e.g. space bar after setting each baud rate.

- If there is no success then change the number of data bits (usually 7 or 8 bits come into question) and try different baud rates again.

- Usually parity and handshake differences should not prevent normal manual terminal use.

- Another method is to switch the counter off and on again.

The correct parameters (terminal type, baud rate and data bits) are set automatically after instrument restart. If they differ from the current parameters then a text like the following is shown: `Incorrect terminal baud rate. 9600 bps used. Please set terminal RS-232C parameters in (S)ystem /(R)S-232C.`

When it seems that communication is working, go to the MicroBeta System-mode and check the serial port 1 settings and make sure that they are identical with settings of the terminal.

Example 6

```
System>R
RS-232C port number                   1->
Baud rate:  1)300  2)600  3)1200
            4)2400 5)4800 6)9600      6->
Parity:  1)None  2)Odd  3)Even        1->
Data bits                             8->
Stop bits                             2->
Handshake:  1)None  2)DTR  3)XON/XOFF 2->
Set terminal port parameters      (Y/N)->Y
Are you sure?                     (Y/N)->Y
Do you see this text (Y/N)?Y
```

2.30.8 (P)rinter

Set the printer type and the number of characters per inch. The options are: IBM or Epson FX and the number of characters per inch 10, 12 or 17. The defaults are Epson FX and 12 cpi.

Example 7

```
System>P

Printer type:    1)IBM  2)EpsonFX           2->

Printer charac.per inch:  1)10  2)12  3)17  2->
```

2.30.9 (D)ata drive

Select diskette or hard disk drive of the terminal PC or MicroBeta on which the results are to be stored (when using UltroTerm define the result file path in UltroTerm Result filing options). The result file path can also be given in each protocol.

Example 8

```
System>D

Data drive:  1)A:  2)B:  3)C:  4)Path   4->

Path name:                 C:\MB\RESULTS\->
```

The options are:
1 = diskette drive A. Select this if you have:
(a) a single drive terminal PC (no other possibilities)
or

(b) a hard disk PC but you want to store results on the diskette (e.g. to be transferred somewhere else).

2 = diskette drive B. Select this if you have a dual diskette drive terminal PC. Then terminal emulator software can be kept in drive A all the time (e.g. to enable automatic restart after power failure).

3 = hard disk drive C. Select this if your terminal PC is equipped with hard disk and you want large storage capacity. If the GenTerm terminal emulator is used, then results will be stored into the directory which was active when GenTerm was started.

4 = full disk drive and directory path. Use this if options 1-3 do not match your requirements. E.g. if you want to store results:

(a) on a hard disk drive other than C: (e.g. D:),
(b) on a local area network (LAN) connected to the terminal PC,
(c) in a specific directory or
(d) on the MicroBeta drive.

If 4 is selected then the program asks for the full drive and directory path. Here understanding of MS-DOS drive and directory concepts is needed. The directory path given here will be used as such without any checking and modifications in front of the file name. Note that the directories must exist before any data can be sent to them. Use the command MKDIR in DOS to make new directories.

Examples (referring to examples a-d above):
(a) Path name: D: ->
- Text D: will be inserted in front of result file name. E.g. D:2.003 , the file will be stored in the active directory on drive D.

(b) Path name: F: ->
- Here it is supposed that drive F: belongs to the LAN server. Text F: will be inserted in front of the result file name. E.g. F:17.002 , the file will be stored in the active directory of the LAN server drive F.

(c) Path name: `C:\MB\RESULTS\` ->
- Text `C:\MB\RESULTS\` will be inserted in front of result file name. Note that if you specify an exact directory path for the result files then the path name must end with backslash \. For example the path `C:\MB\RESULTS\3.023` stores result file `3.023` into the directory `\MB\RESULTS\` of hard disk drive C.

(d) Path name: `@B:\DATA\->`
- Results are stored on the MicroBeta protocol disk B in directory `DATA`. The character @ at the head of the path shows that the MicroBeta disk is to be used for storing the results.

2.30.10 Ter(m)inal

Select the terminal emulation (see chapter 2.31 Terminal emulators for details) that your terminal or terminal PC uses. The additional questions depend on the emulation selected. The possible emulations are:

1 = GenTerm in WT emulation mode. This emulation is fully tailored to be used with MicroBeta, so the only things the user has to specify are (see example 9):

Example 9

```
System>M

Terminal emulation:
1)GenTerm  2)UltroTerm  3)MultiCalc
4)VT52     5)VT100      6)MBW              2->1
Number of lines on screen                 24->
Print through terminal (Y/N)               N->
Do you accept the new terminal setting? (Y/N)->N
```

- the number of lines on the screen.
- whether the printer should be connected to the terminal PC or not (see 2.30.10.5).

2 = UltroTerm (see example 10). Select this if you are using the UltroTerm terminal emulator. UltroTerm V2.0 or later is required.

Example 10

```
System>M

Terminal emulation:
1)GenTerm   2)UltroTerm   3)MultiCalc
4)VT52      5)VT100       6)MBW              2->
Number of lines on screen                   23->
Print through terminal (Y/N)                 N->
Do you accept the new terminal setting? (Y/N)->Y
Prepare your terminal ready for emulation change.

Press any key when ready.
```

3= MultiCalc VT52. Extensions to enable result file saving to PC disk when using MultiCalc.

4 = VT52 (see example 11). Select this if you are using a terminal emulator that can provide only this emulation.

5 = VT100. This emulation is quite popular in commercial terminal emulation programs. If your terminal emulator implements both VT52 and VT100, select VT100 because it supports a better range of MicroBeta features i.e. counting results displaying (screen attributes, result window scrolling etc.).

6 = MBW (MicroBeta Windows workstation VT52). If this is selected then results sorting and live data automatic sending must be specified. The workstation program sets the terminal and other settings automatically.

Additional questions:

2.30.10.1 Number of lines on screen

Possible values from 15 to 25, the default value is 24 lines. Some terminal emulator programs can scroll the whole screen if something is written at the lower right corner of screen (e.g. column 80 on line 24) which mixes the status display. If you encounter this problem give here a smaller value (e.g. 23 lines). You can select a smaller value also if your terminal PC cannot display 24 lines (e.g. some portable PCs can display 16 lines).

2.30.10.2 Block graphics

For VT52 and VT100, default Y=Yes. If your terminal shows some strange characters around the status display and it seems that it cannot display block graphic characters, select N=No.

2.30.10.3 Send result files to terminal

For VT52 and VT100. The method of sending result files to the terminal PC depends on the terminal emulator software. Often a terminal emulator can capture data between a start string and a stop string. If your terminal emulator is capable of doing this then answer Y=Yes. Then MicroBeta stores a result file at first on the protocol diskette and sends it to the terminal as one block as:

```
Start of assay:XX
...
... Result data
...
End of assay
```

where xx is the protocol number.

If your terminal emulator cannot capture data then answer N=No. Then the result files are stored on the protocol diskette of MicroBeta but not transferred to the terminal PC.

Note 1: Result file will be output if protocol line `36 FILE OUTPUT` is set to other than 1 (No output).

Note 2: See chapter Results for details.

2.30.10.4 Automatic result file deletion

For VT52 and VT100, default Y=Yes. The capacity of the protocol diskette is limited (300KB - 1.3MB depending on the number of protocols) and it is capable of storing the results from at least 20 cassettes if the output selection for `FILE OUTPUT` does not specify too many different printout fields. If this selection is set to Y=Yes then the result files on protocol diskette will be deleted automatically when starting counting and after sending a result file to the terminal PC is complete. If you want to ensure that your data has been transferred to another diskette or to some PC then answer N=No. In that case after starting counting the program confirms deletion before operation by asking:

```
Delete result files saved on protocol disk?
```

and you can answer N=No to reject deletion or Y=Yes to accept deletion.

2.30.10.5 Print through terminal

Select here where to connect the printer. Answer:

N=No if you do not need to connect the printer to the terminal PC. Connect instead the printer to serial port 3 of MicroBeta with serial cable 1221-502.

Y=Yes to connect the printer to the terminal PC. In this case connect the printer to the parallel port of the terminal PC with a Centronics cable 1221-122.

2.30.10.6 Exit terminal setting

To exit terminal setting press slash / and Enter on any line. Otherwise, after you have answered all the questions described above, the program confirms that you accept the change. If not, press N, or else press Y which causes the following message to appear:

```
Prepare your terminal ready for emulation change. Press any key when ready.
```

Now you can make the preparations possibly required for the emulation change (e.g. to switch from GenTerm to UltroTerm). When ready press any key. Then if the change succeeded the program returns to the System state. However if the message `Terminal type check failed!` appears (see example 11) it means that MicroBeta has found that the terminal program does not use the same emulation as was selected (MicroBeta sends an identity inquiry to the terminal and the answer sent by the terminal emulator software is different for each emulation -

GenTerm, UltroTerm, VT52, VT100). Answer N if you want to cancel the new terminal emulation. However if you know that your terminal has the correct emulation or you want to switch to the new emulation later you can answer Y.

Example 11

```
System>M

Terminal emulation:
   1)GenTerm 2)UltroTerm 3)MultiCalc
   4)VT52    5)VT100     6)MBW              2->4
Number of lines on screen                   24->
Block graphics (Y/N)                         Y->
Send results file to terminal (Y/N)          N->
Automatic result file deletion (Y/N)         Y->
Print through terminal (Y/N)                 N->
Do you accept new terminal setting? (Y/N)    ->Y
Prepare your terminal for emulation change.
Press any key when ready
Terminal type check failed!
Do you accept the new terminal setting? (Y/N)->N
```

2.30.11 P(a)sswords

Press A to print protocol passwords, entered on line 45 in the protocol editor (see chapter 2.25 Protocols). This is possible only if no System password is in use or in Setup mode (see 2.30.15).

Example 12

```
System>A

Printing protocol passwords, press / to exit
```

2.30.12 Customi(z)ing

Press Z to set environment strings to customize the program. The environment string name and value must be given. For a list of possible strings, see (I)nfo/Customi(z)ing. Giving D as the value sets the default value for the string.

Example 13

```
System>Z

Customized: MENU = N
Environment string name         -> MENU
MENU                            -> D
Environment string name         -> /
```

2.30.13 MS-D(O)S

Press O to get to the MicroBeta MS-DOS. You must confirm this twice as example 14 shows, after which the text MicroBeta A:\> appears:

Example 14

```
System>O

Exit to MS-DOS? (Y/N) ->Y
Are you sure? (Y/N) ->Y

Type MB to start the program again

MicroBeta A:\>MB
(H)elp    (I)nfo
(C)ount   (P)rotocols   (S)ystem

Ready>
```

Type MB and press Enter to get back to the MicroBeta program.

Note: Avoid going to MS-DOS in routine work. The main reasons to go there are to make a backup copy of the protocol or program diskette.

2.30.14 (Q)uit

Press Q to quit from the System state.

2.30.15 Se(t)up mode

If a system password is used then the system mode menu is quite short (see example 15).

Example 15

```
(H)elp       (Q)uit           MS-D(O)S
(E)rror beep (S)tatus display (V)ersion
(C)lock      Se(t)up mode

System>T
Password->
(H)elp       (Q)uit           MS-D(O)S
(E)rror beep (S)tatus display (V)ersion
(C)lock      (R)S-232C        (P)rinter
(D)ata drive Ter(m)inal       P(a)sswords
Customi(i)zing

System>
```

All the functions that can affect routine use are hidden. The person responsible for the MicroBeta system can make the hidden functions visible if he or she knows the password. Press T to get into the setup mode. The program then asks for the password. Type the password and press Enter. The characters you type are not displayed on the screen. If you enter a wrong password the program displays the message:

```
Incorrect password
```

Otherwise the program shows the full System menu.

To set a system password, exit from the MicroBeta program to MS-DOS as described earlier. After the text `MicroBeta A:\>` appears, type `PASSWORD xxxx` and press Enter (xxxx in the above means the new password). If you forget the password then set a new one.

2.31 Terminal emulators

2.31.1 Introduction

MicroBeta can be used with a variety of different terminals and terminal emulator programs. Most of the chapters in part 2 of this manual describe terminal operations. The only exception is when the MultiCalc counter program is used. This chapter describes operation with two general purpose terminal emulator programs from Wallac - GenTerm and UltroTerm, and VT52 and VT100 terminal emulators (see below for an explanation of the terminology). For information about MultiCalc see chapter 2.22 MultiCalc. You can select whichever of these terminal emulations is most suitable for your needs.

2.31.2 Terminology

2.31.2.1 Terminal

This consists of a keyboard, display and electronics, and is not usable as a general purpose computer.

2.31.2.2 Terminal PC

This is any general purpose personal computer that can run a terminal emulator program.

2.31.2.3 Terminal emulator program

This is a program which allows a PC to be used as a terminal and often implements extra features such as data logging, multiple terminal emulations etc.

2.31.2.4 Terminal emulation

This is a control language that can be used to command the terminal to perform simple operations such as clear display, move cursor, use underline etc. The control language consists usually of plain ASCII text, control codes such as backspace and escape sequences (character sequences started by the Esc-character).

Terminal manufacturers have introduced several different terminal emulation languages e.g. VT52, VT100 and Wallac WT.

2.31.2.5 MS-DOS

MS-DOS means Microsoft Disk Operating System (a product of Microsoft Corporation). MS-DOS enables the user to operate on disks, files, directories, execute programs etc. The MicroBeta program itself and usually the terminal emulator program run under MS-DOS. When you exit from the MicroBeta program to MS-DOS which is running in the instrument microcomputer you see the prompt text `MicroBeta A:\>`. In contrast when you exit from the

terminal emulator program to the MS-DOS of the terminal PC the prompt usually shows either the current disk drive (e.g. `A:>`) or the current directory (e.g. `C:\GENTERM>`). For details about using MS-DOS see the MS-DOS User's Manual of your terminal PC.

2.31.2.6 Temporary exit from a terminal emulator program

Temporary exit from a program means that the current program remains in the memory but you can run MS-DOS commands and other (small) programs in the remaining free memory. To return to the original program type EXIT and press Enter.

2.31.2.7 Exit from a terminal emulator program

Exit from a program means that the current program is terminated totally; to return to it you must start the program again.

2.31.2.8 Backup

It is recommended that you make a backup copy of your terminal emulator program diskette(s) before installation. Use the MS-DOS command `DISKCOPY` at the MS-DOS prompt of the terminal PC. See the MS-DOS User's Manual of your terminal PC for details.

2.31.2.9 Start up of the terminal PC

A terminal PC can be started up using one of the following methods:
- switch on power
- if power is on, press the Ctrl, Alt and Del keys simultaneously or press the reset button (this button does not exist in all PC models).

2.31.3 Conventions

Manual references
Interpret notations such as System/Ter(m)inal/Block graphics as:
- chapter = System
- section = Ter(m)inal
- topic = Block graphics

2.31.4 GenTerm

2.31.4.1 Product number and manual

1221-243 GenTerm terminal emulator
1221-921 GenTerm User Manual

2.31.4.2 Version

V2.B or later

2.31.4.3 Installation

(a) For details see the GenTerm User Manual three last pages.

(b) When the installation program asks:

```
Select emulation mode:
1. Normal VT-52 terminal
2. Optimized Wallac Terminal (WT)
```

then answer 2 (WT emulation).

(c) If installation was made on hard disk then select the way how GenTerm should be started as follows (these instructions suppose that GenTerm was installed into directory `C:\GENTERM`):

(1) If GenTerm should start automatically after terminal PC start up, copy lines of `C:\GENTERM\ GTERM.BAT` to the file `C:\AUTOEXEC.BAT` e.g. using the COPY command:

```
COPY C:\AUTOEXEC.BAT+C:\GENTERM\GTERM.BAT C:\AUTOEXEC.BAT
```

(2) Execute GenTerm always from the directory `C:\GENTERM`.

(3) Insert the directory `C:\GENTERM` to the MS-DOS `PATH` variable.

(4) Copy file `C:\GENTERM\GTERM.BAT` into one of the directories listed in the MS-DOS `PATH` variable.

d) In the GenTerm setup menu set the Buffer length to a minimum of 100 characters

2.31.4.4 Starting GenTerm

(a) If installation was made on diskette either:

insert terminal diskette into drive A: and start up the PC

or
type GENTERM at the MS-DOS prompt and press Enter.

(b) If installation was made on hard disk then you have three possibilities to start GenTerm depending on the type of installation:

- Start up the terminal PC (installation (c)(1))

- Type `CD C:\GENTERM` and press Enter then type `GTERM` and press Enter (installation (c)(2))

- Type `GTERM` and press Enter (installation (c)(3) and (4))

2.31.4.5 Changing parameters in GenTerm

The main reason for communication problems is that the counter is configured to use one emulation and the terminal emulator uses some other emulation (see 2.31.4.11 Troubleshooting below). If there still seems to be communication problems after changing to the same emulation in both the counter and the PC the reason can be that there are different communication parameters in the counter and the emulator. Here is a quick guide for how to change the communication parameters in GenTerm. For further information see the GenTerm User Manual.

1. Press the Ctrl and Break keys to get to the GenTerm main menu (or in VT-52 mode press F8).

2. Use the down arrow key to go to Set terminal parameters and press Enter.

3. Select an item from the Set-Up menu by pressing the up or down arrow keys.

4. Change the value of the selected item by pressing the left or right arrow keys.

5. Store the setup on disk as default settings by pressing F3.

6. Press F9 to return to the terminal emulator mode.

Section 2.30 System/(R)S-232C in this manual describes how to change communication parameters in the counter. This section also contains the default parameter settings.

2.31.4.6 Special features

GenTerm WT emulation emulates the IBM ROM BIOS keyboard and display functions. If communication problems arise see the Troubleshooting section below.

2.31.4.7 Restrictions

Do not change GenTerm to ADM-3A or VT52 emulation.

2.31.4.8 Result files

The destination disk drive and directory can be set in System/(D)ata drive or in Protocol/File output.

The MicroBeta program uses a special GenTerm WT emulation to direct output to the display, the printer and the result file. Result file saving is automatic and it is enough for you to only select on the Protocols/File output-line which data should be saved.

2.31.4.9 Printer connection

The MicroBeta program can automatically direct printer output through GenTerm to the printer connected to the terminal PC with a standard Centronics cable. The printer is normally connected to port 3 on MicroBeta.

2.31.4.10 Exit to MS-DOS

To get to the menu press Ctrl-Break or Ctrl-Scroll Lock if the Break-key is missing. Select either Exit to DOS temporarily (temporary exit) or Exit from GenTerm to DOS (total exit), press Enter and follow the instructions. During counting it is recommended that you type D (see chapter 2.16 Interrupt) before temporary exit from GenTerm to MS-DOS. It is then also possible to exit totally from GenTerm and to use the terminal PC for running other programs. After returning to GenTerm press D again to allow MicroBeta to send data to GenTerm.

See the GenTerm User Manual for details of other menu operations.

2.31.4.11 Troubleshooting

(1) Communication problems:
Communication problems (e.g. characters are displayed correctly but the keyboard does not function) will occur if MicroBeta is configured to use GenTerm WT emulation and the terminal emulator uses some other emulation (UltroTerm,VT52,...) or vice versa.

If you suspect that kind of situation then restart -MicroBeta by putting power off and then on again. Terminal type, baud rate and data stop bits are set automatically after instrument restart (see 4.7.2 and 4.7.3). Another method is (if the instrument is in the Ready state) to press several times the key that sends the Escape character to MicroBeta (usually labelled Esc; in UltroTerm F3).

MicroBeta should respond: `Please check terminal type in (S)ystem/Ter(m)inal!.`

After doing this the keyboard will work and you can then set terminal emulation in System/Ter(m)inal. If the message above does not appear then the problem is probably in the serial communication (cable, RS-232C protocol).

(2) Printer errors:
GenTerm shows an error window. Solve the printer problem (power off, off-line mode, paper out etc.) and then press R (Retry) to continue printing. If you cannot fix the problem press A to abort printing and then P until the MicroBeta program asks Pause printing (Y/N). Then answer Y.

(3) Diskette and hard disk problems:
When the diskette or hard disk becomes full, GenTerm shows a message window that enables you to:

(a) Change diskette: insert a new formatted diskette (the result file continues on the new diskette with the same name)

(b) Give a new file name: e.g. direct the result file to another disk drive

(c) Stop logging data

Select the recovery method using the left and right cursor keys, press Enter and follow the instructions.

If the result diskette is missing or is unformatted etc., then GenTerm displays a Drive not ready window. Put a properly formatted diskette into the correct drive and press R to continue result saving. If you cannot solve the problem then press A to stop saving data.

2.31.5 UltroTerm

2.31.5.1 Product number and manual

1221-244 UltroTerm 2 Terminal Emulator
1221-922 UltroTerm 2 User Manual

2.31.5.2 Version

V2.0 or later

2.31.5.3 Installation

(a) For details see the UltroTerm 2 User Manual chapter 3 Installation.

(b) Floppy disk installation: follow steps 1-8 as described in the manual. When you reach step 9, instead type `INSTALLF 1205` and press Enter.

Note: MicroBeta uses the same mode as Wallac 1205 Betaplate. The number 1205 is therefore also used with MicroBeta. The number can be changed in communication parameter setting.

(c) Hard disk installation: follow steps 1-2 and 4-7 as described in the manual, in step 3 instead type `INSTALLH 1205` and press Enter.

(d) If installation was made on hard disk then select the way in which UltroTerm should be started (supposing UltroTerm was installed into directory `C:\UTERM`):

(1) If UltroTerm should start automatically after the terminal PC start up, then copy lines of `C:\UTERM\ START.BAT` to the file `C:\AUTOEXEC.BAT` e.g. using the COPY command:

```
COPY C:\AUTOEXEC.BAT+ C:\UTERM\START.BAT C:\AUTOEXEC.BAT
```

(2) Execute UltroTerm always from directory C:\UTERM.

(3) Insert the directory `C:\UTERM` into the MS-DOS `PATH` variable.

(4) Copy the files `START.BAT, TASK1.BAT` and `BPNAME.EXE` from the directory `C:\UTERM` into one of the directories listed in the MS-DOS `PATH` variable.

Check that `COMMAND.COM` exists in the root directory (C:\) of the terminal PC hard disk.

2.31.5.4 Starting UltroTerm

(a) If installation was made on diskette either

- insert the terminal diskette into drive A: and start up the PC
or
- type START at the MS-DOS prompt and press Enter.

(b) If installation was made on hard disk then you have three possibilities to start UltroTerm depending on the type of installation:

- Start up the terminal PC (installation (d)(1))

- Type CD C:\UTERM and press Enter then type START and press Enter (installation (d)(2))

- Type START and press Enter (installation (d)(3) and (4)).

2.31.5.5 Changing parameters in UltroTerm

The main reason for communication problems is that the counter is configured to use one emulation and the terminal emulator uses some other emulation (see 2.31.5.11 Troubleshooting below). If there still seems to be communication problems after changing to the same emulation in both the counter and the PC, the reason can be that there are different communication parameters in the counter and the emulator. Here is a quick guide for how to change the communication parameters in UltroTerm. For further information see the UltroTerm 2 User Manual.

1. Press the Esc key to get to the UltroTerm main menu.

2. Use the down arrow key to go to Set RS232 comm's protocol and press Enter.

3. Enter communication port number and press Enter.

4. Select an item from the Set-Up menu by pressing the up or down arrow keys.

5. Change the value of the selected item by typing the new value or by selecting from the list using the Home and End keys.

6. Confirm the change by pressing Enter.

7. Store the setup on disk as default settings by answering Y(es) to the last question.

8. Press Esc twice to return to the terminal emulator mode.

Section 2.30 System/(R)S-232C in this manual describes how to change communication parameters in the counter. This section also contains the default parameter settings.

2.31.5.6 Special features

- UltroTerm uses an extended VT52 terminal emulation.

- UltroTerm allows file transfer to a computer, built-in file editing and printing.

2.31.5.7 Restrictions

- UltroTerm can serve only one instrument when in 1205 mode.

In 1205 mode MicroBeta controls printing, so the F7 key does not work.

2.31.5.8 Result files

Select Auto filing start string and Auto filing stop string in Filing parameters. A column selection marker, e.g. # can be used with programmable output, see 2.26.3. See the UltroTerm User Manual for details.

2.31.5.9 Printer connection

The MicroBeta program can automatically direct printer output through UltroTerm to the printer connected to the terminal PC with a standard Centronics cable. The printer is normally connected to port 3 on MicroBeta.

2.31.5.10 Exit to MS-DOS

To get to the menu press the Esc-key. Select either Terminal-DOS gateway (temporary exit) or Exit the program (total exit), press Enter and follow the instructions. When the menu is on the screen press the Esc-key to get back to the terminal mode. During counting it is recommended that the user types D (see chapter 2.16 Interrupt) before temporary exit from UltroTerm to MS-DOS. It is then also possible to exit totally from UltroTerm and to use the terminal PC for running other programs. After returning to UltroTerm press D again to allow MicroBeta to send data to UltroTerm. See UltroTerm 2 User Manual/Menu for details of other menu operations.

2.31.5.11 Troubleshooting

(1) Communication problems

See GenTerm/ Troubleshooting (1)

(2) Printer errors
UltroTerm shows an error window. Solve the printer problem (power off, off-line mode, paper out etc.) and then press the number 1 key to continue printing.

(3) Diskette and hard disk problems
See the UltroTerm User Manual chapter 4.

2.31.6 VT52 and VT100 compatible terminals and terminal emulators

2.31.6.1 Installation

This depends on the product, see the manuals which accompany the product.

For cabling see the chapter 4.1 Installation.

Define the terminal features in System/Ter(m)inal (see below for details and chapter 2.30 System/Terminal).

2.31.6.2 Special features

The terminal understands standard VT52/VT100 escape and other control codes.

2.31.6.3 Restrictions

If the product does not understand VT52/VT100 block graphics characters then set System/Ter(m)inal/Block graphics to N=No.

2.31.6.4 Result files

There are several possibilities:

(1) MicroBeta automatically saves results defined on the Protocol/36 File output line into directory `B:\RESULTS` on the protocol diskette. You can copy files from there if your PC can read standard MS-DOS, 3.5 inch, 1.44MB diskettes.

(2) If you set `System/Ter(m)inal/Send result files to terminal` to `Y=Yes` then MicroBeta sends the result file saved as in (1) at the end of each assay as one block of text in the format:

```
Start of assay:xx
...
...
End of assay
```

where xx is the protocol number. The terminal emulator can now capture the data within Start of assay and End of assay.

(3) It is possible to define the following environment strings in the MicroBeta `AUTOEXEC.BAT`.

```
SET SOPN = <open>   (open result file, name follows)
SET SCLS = <close>  (close result file)
SET SBEG = <begin>  (save characters until <end> is found)
SET SEND = <end>    (end character saving)
```

In the above, <open>, <close>, <begin> and <end> can be any character sequences which do not include the VT52/VT100 control codes and do not appear in the outputs. For the terminal emulator the data coming from the RS-232C port looks as follows:

```
...                              (Display/printer output)
<open><path> xx.yyy <CR>         (Open file xx.yyy in directory <path>)
...
<begin>  POS  CTIME  CCPM1       (save this data into file xx.yyy)
<end>...
...
<begin>  A01     60 102356       (save this data into file xx.yyy)
<end>...
...
<begin>  A02     60 115294       (save this data into file xx.yyy)
<end>...
...
...
<close>                          (close file xx.yyy)
```

where xx=protocol number, yyy=running result file number, <CR>= carriage return character and <path> = drive and directory set in System/(D)ata drive.

2.31.6.5 Printer connection

VT52 and VT100 terminal emulations have control codes to direct output to the printer connected to the terminal. These codes are:

- VT52:
<esc>W (Printer controller mode on)
<esc>X (Printer controller mode off)

- VT100
<esc>[5i (Printer controller mode on)
<esc>[4i (Printer controller mode off)

All characters between printer controller mode on and off codes will be transferred to the printer. All other characters will be displayed. However usually some control character (NULL, backspace etc.) not directed to the printer can disable graphics printing.

2.31.6.6 Troubleshooting

For communication problems see GenTerm/Troubleshooting (1).

2.31.7 UTMAC

2.31.7.1 Product number and manual

1221-170 UTMAC 1.1 Terminal Emulator for Macintosh.
1221-926 UTMAC 1.1 User Manual.

2.31.7.2 Version

V.1.1 or later.

2.31.7.3 Installation

(a) for details see UTMAC User Manual chapter 3 Installation

(b) If you want to run UTMAC automatically on starting up the Macintosh, make an alias of the appropriate UTMAC setup document and put it into the Startup folder.

2.31.7.4 Starting UTMAC

To start UTMAC double-click on the icon of the appropriate setup document or on the icon of the UTMAC application itself. At the present time there is no UTMAC emulation mode in the counter, but UTMAC is 100% compatible with the UltroTerm emulation mode.

2.31.7.5 Changing parameters in UTMAC

The main reason for communication problems is that the counter is configured to use one emulation and the terminal emulator uses some other emulation, so check that the counter is set to UltroTerm terminal emulation mode.

If there still seems to be communication problems after changing to the correct emulation mode, the reason can be that there are different communication parameters in the counter and the emulator. Here is a quick guide for how to change the communication parameters in UltroTerm. For further information see the UTMAC User Manual.

1. Choose Communication from the Settings menu of UTMAC.
2. Modify the appropriate parameters: baud rate, number of data bits, number of stop bits, parity and type of handshake.
3. Press the OK button to confirm the changes.
4. Save the modified parameters in the appropriate setup file using the Save or Save As command from the File menu.

Section 2.30 System/RS-232C in this manual describes how to change communication parameters in the counter. This section also contains the default parameter settings.

2.31.7.6 Special features

UTMAC uses an extended VT52 terminal emulation.

UTMAC allows file transfer to Macintosh and printing.

2.31.7.7 Result files

Select the automatic filing start and stop strings in the Filing dialogue. A column selection marker (data marker) can be used with programmable output, see 2.26.3. See the UTMAC User Manual for details.

Result files will be saved in the same directory where the current UTMAC setup document lies.

2.31.7.8 Printer connection

The MicroBeta program can automatically direct printer output through UTMAC. However, it is not printed immediately but gathered into a temporary printer output file. You can print the output by pressing the Print button that appears at the top of the terminal window or by selecting Print Printer Output from the File menu. After printing, the contents of the temporary file will be deleted. It is recommended that you print the printer output only when the counter is in the Ready state.

2.31.7.9 Exit to Finder

If you are running System 7 or MultiFinder you can always switch to other programs and let UTMAC work in the background. If you want to stop counting temporarily, type D (see chapter 2.16 Interrupt) before switching to some other application. Press D again when back in UTMAC to resume counting.

When you want to exit completely from UTMAC, choose Quit from the file menu. If there is some printer output pending, you will be asked whether or not you would like to print it. If you have modified the setup parameters you will be asked to save them.

2.31.7.10 Troubleshooting

(1) Communication problems
See GenTerm/Troubleshooting (1)

(2) Disk full
UTMAC periodically checks the free space left on volume(s) used for filing and warns you if there is too little space. If any of the volumes gets really full UTMAC halts processing of data sent by counter and displays a warning message. You should switch to Finder and make some space or alternatively you can close the data files. Processing of counting data continues automatically when there is enough free space again or when there are no data files open. Note

however, that you cannot close the temporary printer output file which is always located in the System folder of the boot volume. So if you use the print through terminal option of MicroBeta, make sure that you have enough free space for printer output on the boot volume.

2.32 Thermostat option

When the Thermostat option is installed it allows the temperature of the upper detectors to be reduced and stabilized. This lowers the luminescence counting background because the cooling decreases the photomultiplier tube thermal background. E.g. changing the detector temperature from 27^{o} C to 20 ^{o} C decreases the thermal background by about 60 %.

The thermostat option can be used to cool the upper detectors up to 9 ^{o} C below room temperature.

The detector temperature can be selected in the System-state by using the menu selection '(-)Detector temp'.

System settings with the Thermostat option

```
(H)elp    (I)nfo
(C)ount   (P)rotocols   (S)ystem

Ready>S

(H)elp        (Q)uit              MS-D(O)S
(E)rror beep  (S)tatus display    (V)ersion
(C)lock       (R)S-232C           (P)rinter
(D)ata drive  Ter(m)inal          P(a)sswords
Customi(z)ing (-)Detector temp

System>
```

Example of how to use the Thermostat option

```
System> -

Current detector temperature:  25.2°C
Detector temperature [°C]      24.0->20
Control detector temperature (Y/N)N->Y

Detector cooling can cause water to condense inside the instrument if the
relative humidity is above 73 % !
```

When the detectors are cooled, water can condense inside the instrument depending on room temperature, detector temperature and relative humidity. Please check that the relative humidity is below the number given in the warning text.

Detector temperature can be selected to be part of programmable output by using the heading TD.

2.33 Total count rate

A total count rate register is provided for each of the 7 standard isotopes and one register for the user definable isotope (called Other). At the end of each assay a total accumulated count rate or activity for that assay is printed out. Each of these count rates is then accumulated to form the Total count rate. This is then the total count rate for all assays since the last time the total count rates were reset. You can display the total count rates, print or reset them. You can also define efficiencies for each isotope; these are used to transform count rates into activities. Type T in the Ready or Count state to get to the Total count rate functions. See the example on the next page.

```
Count>T

(H)elp   (Q)uit
(S)how   (P)rint   (R)eset   (D)efine efficiencies

Total countrate>S
Total countrate and activity since Wed 12-July-2000 13:53

Isotope        CCPM       DPM
H-3         32597739         0
I-125              0         0
C-14       124189482   2978592
S-35           86669         0
Cr-51              0         0
P-32               0         0
P-32C              0         0
Other        2014554         0

(H)elp   (Q)uit
(S)how   (P)rint   (R)eset   (D)efine efficiencies

Total countrate>D
Efficiency estimates used (Y/N) N->Y

Isotope     Efficiency
1 H-3            50.00->
2 I-125           0.00->
3 C-14           75.00->90
4 S-35            0.00->
5 Cr-51           0.00->
6 P-32            0.00->
7 P-32C          0.00->
8 Other           0.00->

(H)elp   (Q)uit
(S)how   (P)rint   (R)eset   (D)efine efficiencies

Total countrate>S
Total countrate and activity since Wed 12-July-2000 13:53

Isotope        CCPM       Eff         DPM      nCi     kBq
H-3         32597739      50.0           0    29367    1087
I-125              0                     0        0       0
C-14       124189482      90.0     2978592    62157    2300
S-35           86669                     0        0       0
Cr-51              0                     0        0       0
P-32               0                     0        0       0
P-32C              0                     0        0       0
Other        2014554                     0        0       0

(H)elp   (Q)uit
(S)how   (P)rint   (R)eset   (D)efine efficiencies

Total countrate>
```

2.34 Window settings

2.34.1 Radiation Energy

Radioactive isotopes disintegrate spontaneously. The disintegration results in emissions of alpha, beta or gamma radiation. The radiation type and the energy emitted is specific for each isotope. In the case of beta radiation the emitted beta particles have a wide range of energies, the energy spectrum forming a continuous distribution.

Beta-radiation energy ranging from 0 to 2000 keV can be measured in MicroBeta (the most common beta emitters have their energies between these energy values). The energy scale is converted into a logarithmic scale and divided into 1024 channels. The energy spectrum of a beta emitting isotope falls into a certain segment of the energy scale, called an energy window. A window is a part of the energy scale consisting of many adjacent channels e.g. a window beginning from channel 5 and extending to channel 360 will cover the energy spectrum of tritium in MicroBeta.

2.34.2 Window setting

The MicroBeta LSC has built in windows for the isotopes ^{3}H, ^{125}I, ^{14}C, ^{35}S, ^{33}P, ^{51}Cr, ^{32}P, ^{32}P Cerenkov and for Luminescence counting. The window for Other can be set by the user. The default value is 5-1024. The preset energy windows shown below are used for these isotopes when the isotope is chosen in the protocol. When measuring dual labelled samples any of these window combinations can be used. Note: Before counting, MicroBeta should be normalized for each isotope used.

Isotope	**Channel settings**
^{3}H	5 - 360
^{125}I	5 - 530
^{14}C	150 - 650
^{35}S	150 - 650
^{33}P	150 - 650
^{51}Cr	5 - 170
^{32}P	250 - 960
^{32}P Cerenkov	5 - 1024
Other	5 - 1024
Luminescence	5 - 1024

If only upper or lower photomultiplier tubes are used, then the default window is 150 -1024 for non-luminescence counting.

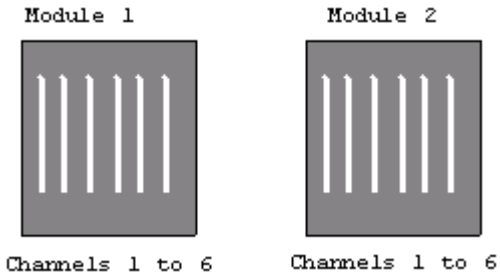
Part 2J Operating information for MicroBeta JET

The information in these chapters is for MicroBeta JET only. For the equivalent MicroBeta TriLux information the chapter with the same number in the preceding part.

2J.0 Injector setup

2J.0.1 Injector modules and channels

When setting up the injectors you are sometimes required to specify the injector module number(s) and the channel number(s). An injector module is a pump with 1, 2, 3 or 6 syringes each feeding tubing that goes to the mask adapter which positions the tips of the tubes above the well(s). Each syringe and hence piece of tubing constitutes one channel. A single detector instrument can have up to four modules each with one channel. A multiple detector instrument can have up to two modules, each with as many channels as there are detectors in the instrument i.e. 2, 3 or 6.



This figure shows the injector system for a six detector MicroBeta JET in which there are two modules each with six channels.

Each module and channel can be switched on or off and thus needs to be identified in parameter setting. This is done by giving the number 0 or 1 in the right position to identify the modules and then 0 or 1, again in the right position to identify the channels. E.g.

```
Injector module number  >1
Use channels            >100100
```

would mean that there is a six detector instrument with a single injector module using channels (syringes) 1 and 4.

```
Injector module number  >2
Use channels            >111111
```

This is a six detector instrument with two modules and all six channels of the second module in use.

2J.0.2 Selecting Injector setup

The MicroBeta JET injectors need setting up before they can be used. This can be done by pressing command letter X when the Counting menu is shown. The injector setup menu then appears as follows:

```
(H)elp (Q)uit
(I)nit (D)ispense  (P)rime    (T)ip
(W)ash Suck-(b)ack Co(m)mand  (S)tatus
O(f)f  (N)ext pos  (G)oto pos (C)learconveyor
Disp(e)nser IN/OUT
```

2J.0.3 (H)elp

Press H to get help about the various selections.

2J.0.4 (Q)uit

Press Q to quit from the Injector menu.

2J.0.5 (I)nit

Press I to initialize the injector module. You must give the number(s) of the injector module(s) to be initialized. Power is switched on to the module and the piston position is reset. This must be the first operation in setup to get the injector system ready.

2J.0.6 (P)rime

This operation fills the tubing with liquid so that there is no dead volume during dispensing. Make sure the tubing is properly connected before this operation. The input tubing should be in the reservoir from where the liquid is coming. Press P to begin this operation. It will take a few seconds. You must give the number(s) of the injector module(s) to be primed and also the numbers of the channels to be used.

2J.0.7 (T)ip

This operation is to make sure that there are no drops hanging from the tips of the injectors. It should be performed after prime has been done. Press T to perform it. You must give the number(s) of the injector module(s) to be tipped and also the numbers of the channels to be used.

2J.0.8 Disp(e)nser IN/OUT

This command should be used to move the mask adapter with its embedded injectors into the counter ready for dispensing to occur. Press E to cause this to happen. This command should be given before the Dispense command described next.

If the mask adapter is already in position in the counter and you give this command then the adapter will be moved out to the injector chamber.

2J.0.9 (D)ispense

Press D to actually cause injection to actually occur. This should not be done until Init, Prime and Tip have been performed. Also the mask adapter should have been moved into place and the plate should be in position. You must give the number(s) of the injector module(s) to be dispensed and also the numbers of the channels to be used. Then give the volume to be dispensed. The units are microlitres. The minimum volume is 10 μL which is also the default. The maximum volume is set by the Dispense volume max. parameter in which the sample volume in the well before dispensing is also taken into account.

For test purposes you can have the mask adapter out and put a waste tray or an empty plate under the injectors to catch the liquid.

2J.0.10 Suck-(b)ack

This command is used to empty the tubing after you have completed dispensing. The liquid will be sucked back into the liquid reservoir, not only emptying the tubing but ensuring that no liquid is wasted. Press B to begin this operation. This operation should be performed before Wash is done.

2J.0.11 (W)ash

This command is used for cleaning the tubing after operation has finished and the tubing has already been emptied of liquid by the Suckback command. Make sure the mask adaptor has been moved out to the injector chamber and a waste tray has been put under the injectors before beginning this operation. The input tubing must be in a reservoir of water or if necessary the cleaning liquid recommended by the manufacturer of the liquid you have been dispensing. Press W to begin this operation. You must give the number(s) of the injector module(s) to be washed and also the numbers of the channels to be used. You must also specify the volume of liquid to be used for dispensing. The units are microlitres and the maximum value is 30,000 μL.

2J.0.12 Co(m)mand

Pressing M allows injector specific macros to be sent to the injector unit. This is mainly for service purposes.

2J.0.13 (S)tatus

If you want to see the status of the injectors, then press S. If there is an error condition you will be informed of it. Otherwise you will be told that the system is ready for operation.

2J.0.14 O(f)f

Press F to switch off the injectors.

2J.0.15 (N)ext pos

When you have the mask adapter and a plate in position you can press N to move the plate to the next position ready for dispensing to the next well.

2J.0.16 (G)oto pos

Press G to move the plate to a selected well position.

2J.0.17 (C)lear conveyor

Press C to move the plate from under the mask adapter back to the cassette rack.

2J.1 Beginning operation of MicroBeta JET

The following conventions are followed all through the MicroBeta user interface:

2J.1.1 Menu selections

Menu selections can be entered when the system is in one of the following states:

```
Ready>                  = Ready for next operation
Protocols>              = Protocol operations
Count>                  = Counting operations
Info>                   = Additional info.
System>                 = System level operations
Counting protocol>      = Counting protocol editor
CPM normaliz. protocol> = CPM norm. protocol editor
DPM standard. protocol> = DPM std. Protocol editor
Assay protocol>         = MultiCalc assay protocol operation
Conveyor>               = Conveyor operation
Total count rate>       = Total count rate manipulation
Injector>               = Injector operations
```

Command options available, e.g. those shown below, are displayed before the prompt text, e.g. Ready:

```
(H)elp          (I)nfo
(C)ount         (P)rotocols     (S)ystem

Ready>
```

The command is executed by pressing the letter (either lower or upper case) enclosed inside parentheses. However commands which include a protocol number (e.g. giving a pure protocol number in Ready state to start counting of that protocol) must be completed by pressing the Enter key.

Help text can be obtained by pressing H, h or ?. Exit to a higher level by pressing Q. Pressing Control-E (the Ctrl and the E keys at the same time) exits always to the Ready state.

2J.1.2 Confirming questions and messages

Quite often MicroBeta displays a message such as:

```
Press any key when ready or / to exit ->
```

This kind of message is used to give to the user time to think if this is really the correct operation and to check that everything is prepared for the operation. Press any key (such as space bar) when ready to continue. Otherwise the user can cancel the operation by pressing slash /.

Messages such as:

```
Do you accept the new terminal setting? (Y/N) ->
```

are used to notify the user that the operation under question can affect the functioning of the instrument, counting results etc. Press Y=Yes to accept the operation or N=No to cancel it.

2J.1.3 Input of data

The rest of the user interface consists mostly of data input. Data input differs somewhat from menu selection and confirming questions:

- The default or the current value is usually displayed before an arrow sign ->.

- After typing the new value you must press Enter.

- Press the Enter key alone to leave the default or current value unchanged.

- Either letter H, h or character ? provide help text about the subject under question. Press Enter after H, h or ?.

- Press slash/ or backslash "**Error! Bookmark not defined.**\" to exit input mode. In the protocol editor / exits with saving the protocol and \ without saving it. Elsewhere usually both cause exit without saving the data.

- Input data can be edited (see below).

2J.1.4 Editing input data

If you make a mistake when entering a new input value you can use the Backspace key (sometimes labelled as BS or a thick left arrow) or the DEL-key to erase the previous character. If the input data is quite long this can be tedious. For this kind of situation a simple keyboard editing facility is provided.

Keyboard editing only uses a few keys so it is easy to learn. The default value or the current value of input data is used as a template for entering a new value. So if you notice that you have made a mistake during an early phase of entering a long input (such as output selection in the protocol editor) you can press Enter to complete entering a new value and then go back to the same line to correct the mistake.

The editing keys are:

<- or Control-B Go back one character in the template (default or current value).

-> or Control-N Go to the next character in the template.

E.g. Control-B is entered by first pressing down the Control key (usually labelled with Ctrl) and then typing letter B (lower case or upper case does not matter). If you keep pressing both the Ctrl-key and letter B down for a while then the repeat function of the terminal sends several Control-Bs to MicroBeta.

Note that Control-B and Control-N move only inside the default or the current value (displayed before ->), not inside a newly typed text.

The cursor control keys (left and right arrows) can sometimes be used instead of Control-B and Control-N. This does not work with every terminal emulator. The up and down arrow keys can be used when moving up and down in the protocol editor or you can type Lx to jump to line number x. Please note that the Enter key must be pressed if making changes to any row.

As an example of keyboard editing, suppose you have entered the following printout selection (the second line):

```
Printer output: POS CTIME CCPM1 CCPM1%
-> POS CTIMR COUNTS1 CCPM1 CCPM1%
```

After you press Enter you will see the text:

```
Error in output heading : CTIMR
```

and an arrow -> will appear. Press the Control-N keys. As you do this, you will see the letters POS CTIMR COUNTS1 etc. appearing. After CCPM1% has been displayed the program will beep to inform that the end of the current value has been reached. Keep Control-B pressed down and you will see that characters at the end of output selection start disappearing. Continue until the cursor blinks just after CTIMR (_ shows the place of the cursor):

```
-> POS CTIMR_
```

If you go too far then stop pressing Control-B and press Control-N to make the lost characters visible again. When the cursor is placed just after `CTIMR` (as in the example) press Backspace or the DEL key once and R will disappear. Type the letter E.

```
-> POS CTIM_            (Backspace or DEL pressed)
```

```
-> POS CTIME_          (Letter E typed)
```

Keep pressing Control-N until there is a beep and you get:

```
-> POS CTIME COUNTS1 CCPM1 CCPM1%
```

You can now continue typing new headings after `CCPM1%`. E.g.:

```
-> POS CTIME COUNTS1 CCPM1 CCPM1% MEAN1 CV1%
```

Now press Enter to complete editing.

If you later want to remove heading `COUNTS1` go to line 31 to get the programmable output selection. Keep pressing Control-N until you are just past `COUNTS1`:

```
-> POS CTIME COUNTS1_
```

Press Backspace or DEL 8 times so that `COUNTS1` and the preceding space disappears:

```
-> POS CTIME_
```

Keep pressing Control-N until there is a beep and press Enter:

```
-> POS CTIME CCPM1 CCPM1% MEAN1 CV1%_
```

You can also add a new heading afterwards. Go to programmable output selection again. Keep pressing Control-N until the cursor is just after `CTIME`:

```
-> POS CTIME_
```

Now type space and the text `SQP(I)`:

```
-> POS CTIME SQP(I)_
```

Keep pressing Control-N until the program beeps and then press Enter:

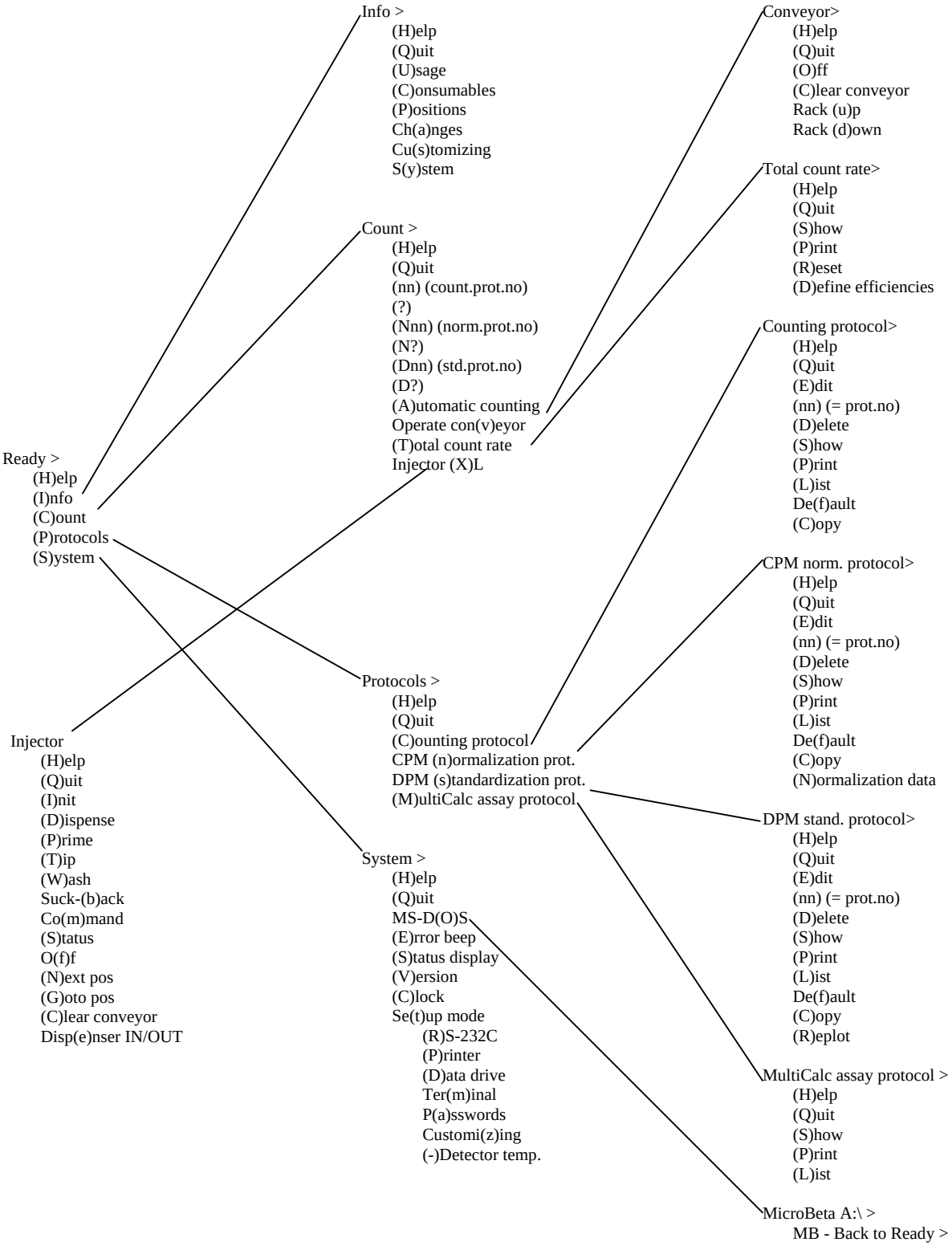
```
-> POS CTIME SQP(I) CCPM1 CCPM1% MEAN1 CV1%_
```

Note: Keyboard editing works with most of the type (2J.1.3) data input

2J.1.5 System operation

The figure following is the flow diagram of the whole MicroBeta operating system. It shows how the control letters inside parentheses lead to various functions. The functions are described briefly on the following pages.

Flow chart of MicroBeta JET commands (normal level)



MicroBeta JET commands (normal level) with a brief description of each

Operations in the Ready State

(H)elp	- Display Help text
(I)nfo	- Get information on using the instrument
(C)ount	- Start counting or operate conveyor
(P)rotocols	- Edit counting parameters
(S)ystem	- Edit system parameters

Count state operations are available in the Ready state too.

Operations in the Info State

(H)elp	- Display Help text
(Q)uit	- Back to the Ready state
(U)sage	- View a short user's manual
(C)onsumables	- View a list of consumables available
(P)ositions	- View normalization and standardization positions
Ch(a)nges	- View a list of changes after the previous version
Cu(s)tomizing	- View information on how to customize the program
S(y)stem	- View information about the system

Operations in the Count State

(H)elp	- Display Help text
(Q)uit	- Back to the Ready state
(nn) (count.prot.no)	- Start automatic counting by giving protocol number
(?)	- Start automatic counting by selecting protocol from list
(Nnn) (norm.prot.no)	- Start CPM normalization by giving protocol number
(N?)	- Start CPM normalization by selecting protocol from list
(Dnn) (std.prot.no)	- Start DPM standardization by giving protocol number
(D?)	- Start DPM standardization by selecting protocol from list
(A)utomatic counting	- Start automatic counting using IDs
Operate con(v)eyor	- Conveyor operations
(T)otal count rate	- Show and reset total counts
(M)anual counting	- Start manual counting (only at Test level)

Operations in the Protocols State

(H)elp	- Display Help text
(Q)uit	- Back to the Ready state
(C)ounting protocol	- Edit counting protocol
CPM (n)ormalization prot.	- Edit CPM normalization protocol
DPM (s)tandardization prot.	- Edit DPM standardization protocol
(M)ultiCalc assay protocol	- Show and print MultiCalc assay protocol

Operations in the Counting Protocol State

(H)elp	- Display Help text
(Q)uit	- Back to the Ready state
(E)dit	- Edit or create and edit a protocol
(nn) (= prot.no)	- Edit a protocol by giving protocol number
(D)elete	- Delete a protocol
(S)how	- Show protocol parameters
(P)rint	- Print a protocol
(L)ist	- Print list of protocols
De(f)ault	- Give default values to a protocol
(C)opy	- Copy one protocol to another

Operations in the CPM Normalization Protocol State

The same operations as in the Counting protocol state, plus:

(N)ormalization data	- Print normalization data

Operations in the DPM Standardization Protocol State

The same operations as in the Counting protocol state, plus:

(R)eplot	- Plot DPM standardization curve

Operations in the MultiCalc Assay Protocol State

Contains only operations (S)how, (P)rint and (L)ist.

Operations in the Conveyor State

(H)elp	- Display Help text
(Q)uit	- Back to the Ready state
(O)ff	- Stop conveyor
(C)lear conveyor	- Move cassette to rack and move rack to the middle
Rack (u)p	- Move the rack up one level
Rack (d)own	- Move the rack down one level

Test level contains also:

Remove ca(s)s	- Move cassette to rack
(N)ext cass	- Move next cassette to conveyor and read ID
(P)rev cass	- Move previous cassette to conveyor and read ID
Cu(r)rent cass	- Move current cassette to conveyor and read ID
Ne(x)t pos	- Move cassette to the next position
(G)oto pos	- Move cassette to a specified position
C(l)ose detector	- Close detector block
Op(e)n detector	- Open detector block

Next le(v)el	- Move rack down to next measurement level
Reset r(a)ck	- Move rack to the highest level
(I)nit mask	- Initialize detector mask (small holes)
Change (m)ask	- Change detector mask
(T)est IDs	- Read and print ID of each cassette continuously
(F)ocus ID reader	- Focus and stop with ID reader on
(J)ust read IDs	- Read IDs continuously and show IDs on the screen
Print ever(y) ID	- Toggle printing of ID bitmaps in ordinary form
Print ra(w) IDs	- Toggle printing of ID bitmaps in raw form
Print (b)ad IDs	- Toggle printing of bad ID bitmaps in raw and ordinary form
Si(z)e checkpoint	- Move cassette to cassette type checkpoint
Calib (0)-pos	- Move cassette between zero point and D06
Calib det bloc(k)	- Move detector block up and down at mask change position
(.)Park det	- Park detector block for shipping
Shelf (1)..(16)	- Move rack to the specified shelf
(*)Calib sensors	- Calibrate sensor offset steps

Operations in the Total Count Rate State

(H)elp	- Display Help text
(Q)uit	- Back to the Ready state
(S)how	- Show the accumulated total count rate and activity
(P)rint	- Print the accumulated total count rate and activity
(R)eset	- Reset the accumulated total count rate and activity
(D)efine efficiencies	- Define counting efficiencies of the isotopes

Operations in the Injector State

(H)elp	- Display Help text
(Q)uit	- Back to the Ready state
(I)nit	- Initialize the selected injector module
(D)ispense	- Volume to be dispensed via the selected tubing of the selected injector module
(P)rime	- Fill the selected tubing of the selected injector module
(T)ip	- Ensure there is no drop hanging from the selected tubing of the selected module
(W)ash	- Wash the selected tubing of the selected module with the selected volume of liquid
Suck-(b)ack	- Suck liquid in selected tubing of the selected injector module back into bottle

Co(m)mand	- Injector specific macros can be sent to the selected injector module
(S)tatus	- Show the status of the selected injector module
O(f)f	- Stop injection
(N)ext pos	- Move the plate to the next position
(G)oto pos	- Move the plate to the selected position
(C)lear conveyor	- Return the plate to the rack
Disp(e)nser IN/OUT	- Move the mask adapter with injectors (injector assembly) in or out

Operations in the System State

(H)elp	- Display Help text
(Q)uit	- Back to the Ready state
MS-D(O)S	- Exit to DOS
(E)rror beep	- Set error sound on or off
(S)tatus display	- Set status display on or off
(V)ersion	- Show version number of program and MS-DOS
(C)lock	- Set date and time
Se(t)up mode	- Select Setup mode

Setup mode contains also:

(R)S-232C	- Set serial port parameters
(P)rinter	- Set printer parameters
(D)ata drive	- Select drive for data saving (results)
Ter(m)inal	- Set terminal parameters
P(a)sswords	- Print protocol passwords
Customi(z)ing	- Set environment strings to customize the program
(-)Detector temp	- Set thermostat (optional)

Test level contains also:

Serial (n)o	- Set instrument serial number
Temp cali(b)	- Edit temperature calibration values
Detector/shelf (u)sage	- Set detector usage during counting, set program support for the ParaLux board and set program support for the plate ID reader if installed and specify 1/16/32 shelf model

2J.6 Counting control

2J.6.1 Counting parameters

The counting process is controlled by the following parameters in the counting protocol:

```
10 Counting time
12 Precision (2 sigma)
41 Counting control
         Number of repeats
         Number of replicates
         Number of cycles
Maximum channel counts (a hidden parameter).
48 Delay between plates
```

Note: Assay protocol parameters are described in chapter 2.26 MultiCalc.

A CPM normalization protocol or DPM standardization protocol does not include parameter 41 Counting control. Instead the crosstalk normalization and crosstalk standardization protocols contain counting time for both crosstalk samples and standards, e.g.:

```
10 Counting time for crosstalk
11 Counting time for standards
```

2J.6.2 Counting time

Counting will be stopped after the specified counting time (in seconds) has elapsed from the start of the counting, unless some other conditions have already stopped it. The maximum counting time is 999 999.9 s (more than 11 days).

2J.6.3 Precision (2 sigma)

The statistical uncertainty in samples is expressed as the standard deviation or 'sigma' value and is calculated by dividing number one by the square root of the counting value. E.g. 10000 counts gives a 'one sigma %' value of 1%. The 2 sigma % value is two times the one sigma % value, or 2% for 10000 counts.

In MicroBeta, precision is given as 2 sigma % in a range from 0 to 99.9. Zero means that no precision test is used.

Make sure that the counting time is set long enough if precision is wanted. E.g. two samples of 1000 and 10000 CPMs will take 10 min and 1 min respectively to achieve a 2 sigma % value of 2%. Precision is checked once a second. The termination flag in results output will be set to

'PREC' if the precision has been reached. The counting stops if the precision has been reached in all detectors.

2J.6.4 Number of repeats

To check sample stability or to check instrument performance, the same sample can be counted repeatedly, up to 999 times. If the repeat value is less than or equal to 5 then results are sorted and some statistical values are calculated and printed after the sample results.

2J.6.5 Number of replicates

To evaluate sample preparation errors, a number of replicates of a sample can be used. The results for each sample are output separately. After each group of replicate samples, some statistical values will be sent to output. The maximum number of replicates is 99.

2J.6.6 Number of cycles

All the sample cassettes in the rack can be counted in repeating cycles. One cycle means counting of all cassettes in a batch once (i.e. those being counted with one protocol). A batch ends when the next cassette with a protocol ID is found. When the instrument has counted all the cassettes in this batch, it moves the rack up until the first cassette in the batch is found. Then it recounts the cassettes in the batch. Counting of the next batch starts when the cassettes have been counted the number of times specified by the Cycle parameter. The repeating cycles can be used to check sample stability. The maximum number of cycles are 99.

If the number of cycles is greater than 1, then you will be asked to give the Cycle delay. This is in minutes and is the time between when one cycle ends and the next one begins.

2J.6.7 Order of operation of repeated counting

Without using injection

If you have set two or all three of the repeat, replicate and cycle parameters to values greater than 1 the order of operation is:

First, repeat counting of each sample. The output consists of the individual results for each repeat count for a sample followed by statistics for the repeat.

Second, statistics for all the replicates of the sample are calculated using the statistics for the repeat.

This procedure is repeated for all samples in an assay batch. When the end of the batch is reached, counting starts from the beginning by counting the repeats and replicates again. This procedure continues for as many times as you have defined for the cycle parameter.

Using injection

When injection is selected it changes the counting procedure so that the positions are dispensed and counted multiple times, depending on the number of Injector Modules used in the protocol. Also the background sample measuring (using the Background sample = Y option) is different, the background is measured for every sample as the first repeat (RP = 0), using the counting time specified for the background sample.

The following example shows the order in which operation occurs.

Dispense with two modules, background sample selected with two cycles, two repeats and two plates:

Plate 1
For all coded positions
  Background
    Count with counting time of background, repeat 0
  Module 1
    Dispense with Module 1
    Wait the delay time of module 1
    Count with counting time of module 1, repeat 1
    Count with counting time of module 1, repeat 2
  Module 2
    Dispense with Module 2
    Wait the delay time of module 2
    Count with counting time of module 2, repeat 1
    Count with counting time of module 2, repeat 2
Wait plate delay time
Plate 2
For all coded positions
  Background
    Count with counting time of background, repeat 0
  Module 1
    Dispense with module 1
    Wait the delay time of module 1
    Count with counting time of module 1, repeat 1
    Count with counting time of module 1, repeat 2

Module 2
Dispense with module 2
Wait the delay time of module 2
Count with counting time of module 2, repeat 1
Count with counting time of module 2, repeat 2
Wait cycle delay time
Cycle 2
Plate 1
For all coded positions
Module 1
Count with counting time of module 1, repeat 1
Count with counting time of module 1, repeat 2
Module 2
Count with counting time of module 2, repeat 1
Count with counting time of module 2, repeat 2
Wait plate delay time
Plate 2
For all coded positions
Module 1
Count with counting time of module 1, repeat 1
Count with counting time of module 1, repeat 2
Module 2
Count with counting time of module 2, repeat 1
Count with counting time of module 2, repeat 2
Note: dispensing and background counting happens only at first cycle.

2J.6.8 Delay between plates

This parameter can be used to control the interval between when the counting of one sample plate has finished and the next is started. The range is 0 to 9999 minutes.

2J.18 Luminescence counting

2J.18.1 Introduction

Luminescence assays come in two main forms; those making use of luminescence that has a short signal (flash luminescence) and those making use of that which emits a long stable signal (glow luminescence). Both can be measured with MicroBeta, however the short emission assays require reagent addition, mixing and temperature control. This means that you must use MicroBeta JET with the injector module(s) installed.

Assays with a more stable luminescence chemistry can be measured with MicroBeta TriLux or MicroBeta JET without using injection. These luminescence assays are designed to be completed on a solid support e.g. nylon membrane, glass fibre or coated plate, as well as in solution and can be directly quantified in a short time.

Due to the high signal, glow luminescence reactions can be measured using single PMTs. An optional mask adapter can be moved into place to reduce the signal by a factor of 10 if required.

The traditional microtitration plates are totally opaque (black or white). These plates are counted with the upper tube and a 1450-105 cassette is used. The filters are counted with the upper or lower tube and a 1450-104 filter cassette is used.

A special feature when using MicroBeta as a glow-type luminometer is the 1450-466 coloured sealing tape. The saturation of PMTs is prevented and crosstalk between adjacent positions is minimized by cutting the intensity of luminescence light with the 1450-466 coloured sealing tape.

The luminescence units used are LCPS: luminescence counts per second i.e. corrected CPS-values/100. Before counting the actual samples, the detector and hence the LCPS-values should be normalized with a luminescence solution as similar to the actual samples as possible. The normalization is done first and it is made use of when counting the actual samples.

2J.18.2 Preparation of glow-type normalization samples (96 and 384-well format)

Make a sample solution which corresponds to the samples to be analyzed, i.e. has the same enzyme and substrate and pipette the same volume as in the samples, into well/membrane G11 (96-well, 1-6 det.), N22 (384-well, 1-6 det.).

An empty background plate is used for background measurement

The 96-well sample array is as follows, where S1 is the sample solution:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G											S1	
H												

Plate

Close the sample plate with 1450-461 sealing tape or with 1450-466 coloured sealing tape and place on a 1450-105 cassette for counting.

Filter

Enclose the filter in a 1450-432 plastic sample bag and place it on a 1450-104 cassette; use 1450-466 coloured sealing tape between the sample bag and detector.

2J.18.3 Preparation of glow-type normalization samples (24-well format)

The procedure is the same as with a 96-position sample support, but the normalization sample position is D5 for S1.

	1	2	3	4	5	6
A						
B						
C						
D					S1	

2J.18.4 Preparation of flash-type normalization samples (96-well format)

Standards are arranged in blocks on the normalization plate. If background sample measurement is used then the background sample must be in the first position in each block on the plate, see the plate maps following.

The plate maps for different detector configurations with 96-well plates and the maximum number of replicates are shown below. If there is less than the maximum number of replicates, the replicates positions are from left-to-right and top-to-bottom.

3 and 6-det. models without background samples. Rows E-H are empty in the 3-det. model.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	S1	S1	S2	S2	S2	S2	S3	S3	S3	S3
B	S1	S1	S1	S1	S2	S2	S2	S2	S3	S3	S3	S3
C	S1	S1	S1	S1	S2	S2	S2	S2	S3	S3	S3	S3
D	S1	S1	S1	S1	S2	S2	S2	S2	S3	S3	S3	S3
E	S4	S4	S4	S4	S5	S5	S5	S5	S6	S6	S6	S6
F	S4	S4	S4	S4	S5	S5	S5	S5	S6	S6	S6	S6
G	S4	S4	S4	S4	S5	S5	S5	S5	S6	S6	S6	S6
H	S4	S4	S4	S4	S5	S5	S5	S5	S6	S6	S6	S6

3- and 6-det. models with background samples. Rows E-H are empty in the 3-det. model.

	1	2	3	4	5	6	7	8	9	10	11	12
A	**B1**	S1	S1	S1	**B2**	S2	S2	S2	**B3**	S3	S3	S4
B	S1	S1	S1	S1	S2	S2	S2	S2	S3	S3	S3	S3
C	S1	S1	S1	S1	S2	S2	S2	S2	S3	S3	S3	S3
D	S1	S1	S1	S1	S2	S2	S2	S2	S3	S3	S3	S3
E	**B4**	S4	S4	S4	**B5**	S5	S5	S5	**B6**	S6	S6	S6
F	S4	S4	S4	S4	S5	S5	S5	S5	S6	S6	S6	S6
G	S4	S4	S4	S4	S5	S5	S5	S5	S6	S6	S6	S6
H	S4	S4	S4	S4	S5	S5	S5	S5	S6	S6	S6	S6

1- and 2-det. models without background samples. Rows E-F are empty in the 1-det. model.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1
B	S1	S1	S1	S1								
C												
D												
E	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2
F	S2	S2	S2	S2								
G												
H												

1- and 2-det. models with background samples. Rows E-F are empty in the 1-det. model.

	1	2	3	4	5	6	7	8	9	10	11	12
A	**B1**	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1
B	S1	S1	S1	S1								
C												
D												
E	**B2**	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2
F	S2	S2	S2	S2								
G												
H												

2J.18.5 Preparation of flash-type normalization samples (24-well format)

Plate maps for different detector configurations with 24-well plates and the maximum number of replicates are shown below. If there is less than the maximum number of replicates, the replicates positions are from left-to-right and top-to-bottom.

3- and 6-det. models without background samples. Rows C-D are empty in the 3-det. model.

	1	2	3	4	5	6
A	S1	S1	S2	S2	S3	S3
B	S1	S1	S2	S2	S3	S3
C	S4	S4	S5	S5	S6	S6
D	S4	S4	S5	S5	S6	S6

3- and 6-det. models with background samples. Rows C-D are empty in the 3-det. model.

	1	2	3	4	5	6
A	**B1**	S1	**B2**	S2	**B3**	S3
B	S1	S1	S2	S2	S3	S3
C	**B4**	S4	**B5**	S5	**B6**	S6
D	S4	S4	S5	S5	S6	S6

1- and 2-det. models without background samples. Row C is empty in the 1-det. model.

	1	2	3	4	5	6
A	S1	S1	S1	S1		
B						
C	S2	S2	S2	S2		
D						

1- and 2-det. models with background samples. Row C is empty in the 1-det. model.

	1	2	3	4	5	6
A	**B1**	S1	S1	S1		
B						
C	**B2**	S2	S2	S2		
D						

2J.18.6 Normalization protocol

Edit the normalization protocol so that it is suitable for luminescence measurements. To do this in the Ready-state press P (P)rotocol then N (N)ormalization protocols. Select edit (E) and the protocol number.

Edit the protocol parameters. See chapters 2.23 and 2J.23 for examples of normalization parameters. Select luminescence (9) on line 5, isotope 1, and the counting time on (line 10). Typically this is 1 s. If required, you should also select the mask adapter and if you want injection then the number of injector modules must be given (i.e. not zero). These are both part of the settings for parameter 5.

For glow-type luminescence a background plate is counted only if selected on line 42.
For flash-type luminescence using injectors a background plate is not used but instead, if required, background samples as described in sections 2J.18.4 and 5 above.

Make sure the injectors are ready for operation as described in chapter 2J.0 Injector operation.

2J.18.7 Normalization counting

First place the empty background plate in a cassette (if you are using such a plate)
Place the plate with the normalization samples in a cassette that is identified with the corresponding normalization protocol number in the protocol number area and with a NORM label in the function code area.

Insert the cassette(s) into the rack and close the door. Press either A (Automatic counting), or Nxx, where xx is the normalization protocol number, in the Ready or Count state to start counting.

If Nxx is selected the program asks:

```
Do you want to continue counting after normalization? (Y/N)->
```

If Y (Yes) is selected, the counting continues after counting the normalization plate, if N (No) is selected the counting stops after the normalization. If normalization has already been done for the protocol then the program asks first:

```
This protocol contains normalization data.
Continue? (Y/N) ->
```

This is to prevent accidental overwriting of normalization data.

If automatic counting is selected the normalization cassette can be placed anywhere in the rack. The NORM label is needed in this case.

The counting results for the normalization are output as specified on lines 30 - 37 in the normalization protocol, and after them the background and detector efficiency values are given.

2J.18.8 Counting luminescence samples

Edit a counting protocol:

In the Ready state select P (P)rotocol then C (C)ounting protocols.

Then select edit (E) and protocol number.

On parameter line 2 select 1 (CPM) and on line 3 select the number of the luminescence normalization protocol defined above. On line 42 select Y (Background correction) and on the next line Y (Use normalization background). Edit the other parameters normally.

Start counting in the Ready or Count state using automatic counting and a protocol ID label or start with the protocol number and press Enter.

When counting the samples, the CPS-values are shown on the status display. The stored normalization factors are used for correcting the LCPS-values in the printout. The normalized LCPS-values are shown after counting on the status display.

2J.23 Normalization

2J.23.1 What is normalization?

MicroBeta JET has 1, 2, 3, or 6 detectors allowing it to count the respective number of samples simultaneously. In order for the results from each detector to be equivalent, irrespective of small variations in efficiency and background between detectors, it is necessary to determine the relative efficiency and background of each detector and then correct for it. This is called normalization.

Normalization without injection

In the case when the injection modules are not used, normalization is done by measuring an optional background plate followed by one or two standard samples with defined activity or CPM in each detector. Once CPMs have been measured the relative efficiencies can be calculated. The efficiency of the detector giving the highest count rate is then defined to be one and the other detector efficiencies are expressed as a fraction of this value. These fractions are called efficiency coefficients.

If isotope activity is given, then absolute efficiencies are calculated by dividing the count rates by the activity. In this way, sample quench and detector absolute efficiencies can be corrected for.

When measuring a sample with a particular detector, the CPM of the sample is corrected by dividing the CPM by the efficiency coefficient. In dual label counting, corrections are made using dual label correction formulae (see 3.3 Calculation methods).

When normalization is done the results are stored with the normalization protocol. The normalization data can be used by one or several protocols and is selected when editing the protocol (see 2J.25 Protocols).

Normalization with injection

The principle is the same as normalization without injection but there is a difference in the arrangement of the background and standards. In addition parameter line 5 must be: `9) Luminescence`. With other selections the Mask Adapter can be selected but not injection. Instead of a background plate, a background sample is used for each detector. The position of the background sample and standards is shown in the plate maps in section 2J.23.4.

2J.23.2 When is normalization necessary?

Each counting or assay protocol requires normalization data to be stored before it can calculate corrected CPMs for the samples counted. A fresh normalization must be done when a new

isotope is counted or when counting features, such as isotope or window, are changed. When a new type of microtitration plate is used, crosstalk normalization may be necessary.

2J.23.3 Editing normalization parameters

Normalization parameters are stored in normalization protocols. These parameters are shown in the figure for the case in which injection is selected i.e. luminescence and mask adapter are both selected.

Note: If you do not want injection then set the dispensing modules parameter to zero. Otherwise set the number of dispensing (injector) modules using the code described on the next page:

```
Line (1..46, type / to exit, ? for help)   1 ->
 1 Protocol name:
   ->flash lumi norm
   Normalization not done
 3 Crosstalk correction (Y/N)              N ->
 4 Number of labels:   1)Single  2)Dual    1 ->
 5 Isotope 1:  1)H-3    2)I-125  3)C-14
               4)S-35   5)Cr-51  6)P-32
               7)P-32 Cerenkov   8)Other
               9)Luminescence              9 ->
     PMT use:  1)Normal  2)Upper  3)Lower  2 ->
     Window 1:                        5-1024
     Use mask adapter  (Y/N)               N ->Y
     Dispensing:  1)High 2)Low 3)Custom    1 ->
     Dispensing modules:                  11 ->01
 7 Sample volume [uL]                    100 ->
 8 Dispensing volume [uL]                 10 ->
 9 Delay time after dispensing [s]       1.0 ->
10 Counting time (s)                     1.0 ->
12 Precision (2 sigma) [%]               0.2 ->
30 Printer output:  1)No    2)Short
                    3)Long  4)Programmable 3 ->
32 Display output:  1)No    2)Short
                    3)Long  4)Programmable 3 ->
34 External output: 1)No    2)Short
                    3)Long  4)Programmable 1 ->
36 File output:     1)No    2)Short
                    3)Long  4)Programmable 1 ->
40 Change special features  (Y/N)          N ->Y
41   Number of replicates                  1 ->
42   Background sample (Y/N)               N ->
       Background counting time [s]     60.0 ->
43   Half-life correction  (Y/N)           N ->
45   Use password  (Y/N)                   N ->
46   Special plate:  1)Wallac  2)BP filter
                     3)Other               1 ->
```

One module or only the first module	= 1
The second of two modules	= 10
Both of two modules	= 11
The third of three modules	= 100
The first and third of three	= 101
etc up to	
All four of four modules	= 1111.

There can be a maximum of 100 normalization protocols, numbered from 0 to 99. Protocol number 0 is the default protocol.

Protocols 91 to 98 inclusive are made at the factory for isotopes ^{3}H, ^{125}I, ^{14}C, ^{51}Cr, ^{32}P, dual label $^{3}H/^{14}C$, ^{35}S, and ^{32}P Cerenkov respectively. These may not be deleted because they are connected to the corresponding counting protocols. Note that no normalization is done for those protocols. You must use these protocols to do the normalization before they can be used for counting unknowns.

Press P and N in the Ready state to choose the normalization protocol state. The protocols can then be edited, copied, deleted, printed and displayed. Press F to get default parameters and E to edit a protocol. C is used for copying a protocol including normalization data and D for deleting a protocol.

Note: the normalization data will be destroyed when editing an old normalization protocol. The normalization must therefore be done again for this protocol. Only the protocol name and password can be edited without destroying normalization data.

See chapter 2J.25 Protocols for further details on normalization parameters and editing.

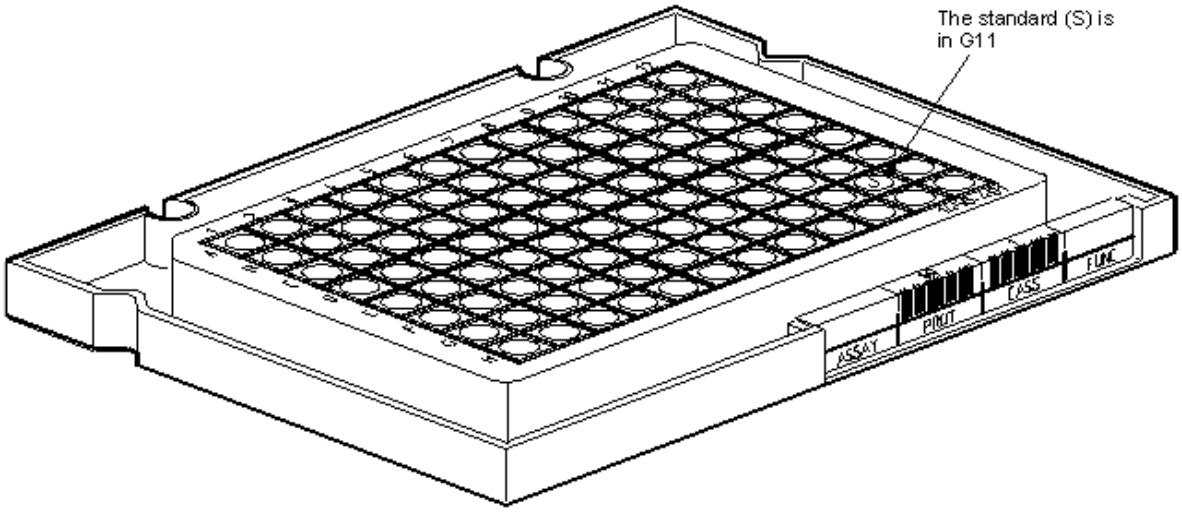
2J.23.4 Preparing the normalization samples

When injection is not used

Depending on which type of MicroBeta you have it is supplied from the factory with:

(for 1-6 detector instruments with 24 or 96-well capability) a 24-well normalization sample plate containing standards for ^{3}H, and ^{14}C. The ^{3}H standard is in D5 and the ^{14}C standard is in D6.

The sample positions and the activities for the standards are shown on a label on the plate. The activity values can be inserted into the normalization protocol.



Sample position in a normalization plate

Be careful that the samples are in the correct positions when making your own normalization plates. The positions cannot be changed by modifying protocol parameters. In 24-well plates the standard for isotope 1 is in D5 and the standard for isotope 2 is in D6. Isotope 1 is the lower energy isotope in dual label counting. In single label counting the standard is in the isotope 1 position, i.e. D5, with one exception:

the standard for ^{14}C must be in the isotope 2 position in single label counting with 24-well plates.

This is because the factory made normalization plate could be used for both single label ^{3}H and ^{14}C normalizations and dual label $^{3}H/^{14}C$ normalizations.

For best results, 96-well plates should be used as normalization plates if 96-well sample plates are to be counted in a 1-6 detector counter. The isotope 1 standard should be in G11 and the isotope 2 standard in H12.

See 2.7.2 Crosstalk CPM normalization for crosstalk sample positions and 2J.18 for luminescence sample positions.

If background sample is selected on line 42, then thermal backgrounds for count subtraction are measured automatically. An empty background cassette must be the first normalization cassette followed by the normalization cassette with standards.

See the table below for sample positions in normalization plates:

Sample position in a normalization plate		
	24-well	96-well
	(1-6 det.)	(1-6 det.)
Isotope 1 standard	D5	G11
Isotope 2 standard	D6	H12
^{14}C single label	D6	

When injection is used

Standards are arranged in blocks on the normalization plate. If background sample measurement is used then the background sample must be in the first position in each block on the plate, see the plate maps following.

The plate maps for different detector configurations with 96-well plates and the maximum number of replicates are shown below. If there is less than the maximum number of replicates, the replicates positions are from left-to-right and top-to-bottom.

3- and 6-detector models without background samples. The rows E-H are empty in the 3-detector model.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	S1	S1	S2	S2	S2	S2	S3	S3	S3	S4
B	S1	S1	S1	S1	S2	S2	S2	S2	S3	S3	S3	S3
C	S1	S1	S1	S1	S2	S2	S2	S2	S3	S3	S3	S3
D	S1	S1	S1	S1	S2	S2	S2	S2	S3	S3	S3	S3
E	S4	S4	S4	S4	S5	S5	S5	S5	S6	S6	S6	S6
F	S4	S4	S4	S4	S5	S5	S5	S5	S6	S6	S6	S6
G	S4	S4	S4	S4	S5	S5	S5	S5	S6	S6	S6	S6
H	S4	S4	S4	S4	S5	S5	S5	S5	S6	S6	S6	S6

3- and 6-detector models with background samples. Rows E-H are empty in the 3-detector model.

	1	2	3	4	5	6	7	8	9	10	11	12
A	**B1**	S1	S1	S1	**B2**	S2	S2	S2	**B3**	S3	S3	S3
B	S1	S1	S1	S1	S2	S2	S2	S2	S3	S3	S3	S3
C	S1	S1	S1	S1	S2	S2	S2	S2	S3	S3	S3	S3
D	S1	S1	S1	S1	S2	S2	S2	S2	S3	S3	S3	S3
E	**B4**	S4	S4	S4	**B5**	S5	S5	S5	**B6**	S6	S6	S6
F	S4	S4	S4	S4	S5	S5	S5	S5	S6	S6	S6	S6
G	S4	S4	S4	S4	S5	S5	S5	S5	S6	S6	S6	S6
H	S4	S4	S4	S4	S5	S5	S5	S5	S6	S6	S6	S6

1- and 2-detector models without background samples. Rows E-F are empty in the 1-detector model.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1
B	S1	S1	S1	S1								
C												
D												
E	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2
F	S2	S2	S2	S2								
G												
H												

1- and 2-detector models with background samples. Rows E-F are empty in the 1-detector model.

	1	2	3	4	5	6	7	8	9	10	11	12
A	**B1**	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1
B	S1	S1	S1	S1								
C												
D												
E	**B2**	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2
F	S2	S2	S2	S2								
G												
H												

Plate maps for different detector configurations with 24-well plates and the maximum number of replicates are shown below. If there is less than the maximum number of replicates, the replicates positions are from left-to-right and top-to-bottom.

3- and 6-detector models without background samples. Rows C-D are empty in the 3-detector model.

	1	2	3	4	5	6
A	S1	S1	S2	S2	S3	S3
B	S1	S1	S2	S2	S3	S3
C	S4	S4	S5	S5	S6	S6
D	S4	S4	S5	S5	S6	S6

3- and 6-detector models with background samples. Rows C-D are empty in the 3-detector model.

	1	2	3	4	5	6
A	**B1**	S1	**B2**	S2	**B3**	S3
B	S1	S1	S2	S2	S3	S3
C	**B4**	S4	**B5**	S5	**B6**	S6
D	S4	S4	S5	S5	S6	S6

1- and 2-detector models without background samples. Row C is empty in the 1-detector model.

	1	2	3	4	5	6
A	S1	S1	S1	S1		
B						
C	S2	S2	S2	S2		
D						

1- and 2-detector models with background samples. Row C is empty in the 1-detector model.

	1	2	3	4	5	6
A	B1	S1	S1	S1		
B						
C	B2	S2	S2	S2		
D						

2J.23.5 Normalization procedure

-First place the empty background plate in a cassette (if you are using such a plate) and then place the plate with the normalization samples in a cassette that is identified with the corresponding normalization protocol number in the protocol number area and with a NORM label in the function code area. Insert the cassette into the rack and close the door. Press either A (Automatic counting), or Nxx, where xx is the normalization protocol number, in the Ready or Count state to start counting. If Nxx is selected the program asks:

```
Do you want to continue counting after normalization? (Y/N)->
```

If Y (Yes) is selected, the counting continues after counting the normalization plate, if N (No) is selected the counting stops after the normalization. If normalization has already been done for the protocol then the program asks first:

```
This protocol contains normalization data.
Continue? (Y/N) ->
```

This is to prevent accidental overwriting of normalization data.

If automatic counting is selected the normalization cassette can be placed anywhere in the rack. The NORM label is needed in this case.

The counting results for the normalization are output as specified on lines 30 - 37 in the normalization protocol, and after them the background and detector efficiency values are given.

Note! Normalization and unknown samples should have the same quench level in dual label counting.

2J.23.6 Preset normalizations

There are four preset normalizations available in MicroBeta:

Normalization protocol 1 contains a ^{3}H normalization using ScintiStrip plate 1450-419, dry, 200 μL volume with crosstalk correction.

Normalization protocol 2 is the same as 1 but using ^{125}I.

Normalization protocol 3 contains a generally used ^{3}H normalization.

Normalization protocol 4 contains a generally used ^{14}C normalization.

2J.25 Protocols

2J.25.1 Four types of protocols

A protocol is a set of parameters that controls the counting of samples. There are four types:

- CPM normalization protocol
- DPM standardization protocol
- MicroBeta Counting protocol
- MultiCalc Assay protocol

A CPM normalization protocol contains parameters used when doing normalization including when using the JET features (see chapter 2J.23 Normalization) and a DPM standardization protocol contains parameters for standardization (see chapter 2.12 DPM Standardization). Standardization is not used with the JET feature. A MicroBeta counting protocol is used when counting unknown samples. An assay protocol is the corresponding protocol in MultiCalc (see MultiCalc). A counting protocol uses either a normalization protocol (in CPM counting) or a standardization protocol (in DPM counting) to get certain parameters, e.g. isotopes and window settings. Assay protocols are connected to counting protocols, from which they get both basic parameters, such as isotopes and counting mode, and special features, such as background and half-life corrections.

The maximum number of protocols for each type is 100 (0 to 99). Number 0 is the default protocol, which is used if no protocol number is specified.

Counting protocol number 99 is a so-called GLP (Good Laboratory Practice) protocol. Count the normalization sample plate supplied with the instrument using this protocol. Do this from time to time to make sure that the instrument produces consistent results. The background, ^{3}H and ^{14}C samples are counted with all detectors so background level, CPM values and quench parameter values can easily be compared. See chapter 3.2 Routine maintenance for more details.

In this chapter only MicroBeta protocols are described. For further information about assay protocols see chapter 2.22 MultiCalc and the MultiCalc User manual Protocols.

2J.21.2 Listing protocols

To list or edit protocols, press P and then C for counting protocols, N for normalization protocols or S for standardization protocols in the Ready state. To select the MultiCalc assay functions as described in chapter 2.22 press M.

To display the protocol list, in the Protocols menu press S (Show). You will see a two column list of protocols containing protocol number, name (if given) and isotopes. The list of the counting protocols also contains counting mode (CPM or DPM) and the number for the normalization or standardization protocol used. The lists of the normalization and standardization protocols have a column containing the date when the normalization or standardization has been done. An equals (=) character indicates that crosstalk correction is used.

If there are more protocols than there is space for on the display, press Enter to get the next page of protocols. When you have found the one you want to look at, type the number of that protocol and press Enter.

Type L (List) to printout the protocol list and P (Print) followed by protocol number and Enter to print a particular protocol.

2J.25.3 Creating and editing protocols

To create a new protocol with default values, press E (Edit). Choose a protocol number not in the list and press Enter. The first free protocol number is shown in the menu. Protocols have already some values in them, either default or old parameters. If you want to set default parameters, press F (deFault) and select the protocol number.

You will be asked for a line number when starting editing a protocol. Press Enter to start editing from the beginning of the protocol or type a line number followed by Enter to edit a certain protocol line. You can always jump between protocol lines by pressing L followed by a line number and Enter. Type P and press Enter to jump to the previous line. The up and down arrow keys can also be used.

The value in front of the arrow on each line is the default value. Just press Enter if you do not want to change this value, or type a new value and press Enter if it must be changed. The Ctrl-B (Ctrl key held down and the B key pressed) and Ctrl-N keys can be used to move within the input text; the left and right arrow keys can also be used. See 2J.1.4 Editing input data.

NOTE: Unnecessary lines in a protocol can be hidden to make the editor shorter. Press Ctrl-K (Ctrl and K keys pressed at the same time) to hide the current line. To reveal a hidden line, go to the next line and press Ctrl-U. To unhide all hidden lines go to line selection line in the beginning of the editor and press Ctrl-A.

Press / and Enter when you are finished with your editing and want to save the changes made. If you want to exit without saving the new values, press \ and Enter.

2J.25.4 Example protocol listing

The example alongside shows a luminescence protocol listing. The parameters are described in section 2J.25.7.

```
Counting protocol no: 26 Tue 18-July-2000 10:49
Name: Flash lumi
CPM normalization protocol no:2
Wallac 1450 MicroBeta V4.4 S/N 4500759
 1 Protocol name:                        Flash lumi
 2 Counting mode:   1)CPM  2)DPM                  1
 3 CPM normalization protocol                     -
 4 Number of labels:  1)Single  2)Dual            1
 5 Isotope 1:  1)H-3     2)I-125      3)C-14
               4)S-35    5)Cr-51      6)P-32
               7)P-32 Cerenkov        8)Other   9
     PMT use:  1)Normal    2)Upper     3)Lower  2
     Window 1:                             5-1024
     Use mask adapter (Y/N)                       Y
     Use injector (Y/N)                           Y
     Dispensing:  1)Automatic    2) Manual        1
     Dispensing:  1)High  2)Low  3) Custom        1
     Dispensing modules                          11
 7 Sample volume [uL]                           100
 8 Dispensing volume 1 [uL]                      10
   Dispensing volume 2 [uL]                      10
 9 Delay time after dispensing 1 [s]           1.0
   Delay time after dispensing 2 [s]           1.0
10 Counting time 1 [s]                         1.0
   Counting time 2 [s]                           1
12 Precision (2 sigma) [%]                     0.2
20 Count all positions in cassette (Y/N)         Y
23 Plate orientation  1)Normal   2)Rotated
                      3)Mirrored 4)Both          1
30 Printer output:  1)No      2)Short
                    3)Long    4)Programmable     3
32 Display output:  1)No      2)Short
                    3)Long    4)Programmable     3
34 External output: 1)No      2)Short
                    3)Long    4)Programmable     1
36 Fileoutput:      1)No      2)Short
                    3)Long    4)Programmable     1
40 Change special features (Y/N)                 Y
41 Counting control (Y/N)                        Y
     Number of repeats                           1
     Number of replicates                        1
     Number of cycles                            1
42 Background correction (Y/N)                   Y
     Use normalization background (Y/N)          N
     Background sample (Y/N)                     Y
       Bgnd counting time [s]                  1.0
43 Half-life correction (Y/N)                    N
45 Use password (Y/N)                            N
48 Delay between plates [m]                      0
```

2J.25.5 Protocol copy and delete

The contents of one protocol can be copied to another by pressing C (Copy). Select the No. of the protocol to be copied and enter the new protocol number. If you want to delete a protocol, press D (Delete) and select the protocol number. Press Q (Quit) to return to the Ready state.

2J.25.6 Protecting protocols

Protocols can be protected against unauthorized editing by using passwords. The password is selected on line 45 in the protocol editor. This prevents other users from changing or deleting your protocol, but they can still use it (e.g. listing, copying and counting with it). The MicroBeta supervisor has the authority to list the passwords.

2J.25.7 Parameters in a counting protocol

1. Protocol name

Type a name for the protocol, maximum 170 characters (only the first 16 characters will be shown in the protocol list) and press Enter. The name may not start with characters ?, / or \.

2. Counting mode

Type 1 for CPM counting or 2 for DPM counting.

3. CPM normalization protocol / DPM standardization protocol

Type the number for the normalization (in CPM counting) or standardization protocol (in DPM counting) used. Type H or ? to get a list of normalization/standardization protocols. Isotopes must be entered if no protocol is selected, otherwise the counting protocol uses the same isotope(s) as in the norm./std. protocol. Type - if no protocol is needed. This means that no normalization correction is done in CPM counting and no DPMs are calculated in DPM counting. Lines 4 and 5 are displayed only if no normalization or standardization protocol number is selected. Information about whether norm./std. has been done is given after line 3.

4. Number of labels

Type 1 for single label counting (one isotope, default) or 2 for dual label counting (two isotopes).

5. Isotope 1

Select an isotope, in dual labelled samples the isotope with the lower energy. The alternatives are:

1) ^{3}H (default) 2) ^{125}I
3) ^{14}C 4) ^{35}S
5) ^{51}Cr 6) ^{32}P
7) ^{32}P Cerenkov 8) Other
9) Luminescence

You will be asked for window limits if you select 'Other', otherwise window settings for the selected isotope are shown.

ParaLux counting can be selected in single label non-crosstalk and non-luminescence protocols. If ParaLux is selected then AQP(I) is used instead of SQP(I). ParaLux counting has two modes: High efficiency and Low background. The discriminator channel that is used to calculate AQP(I) and High efficiency counts can also be optimized.

For PMT use select normal coincidence counting, or use only upper or lower photomultiplier tubes. Upper is the default if luminescence counting is selected.

Window 1 shows what the window setting is. If 'Other' has been selected as isotope 1 enter the limits for window 1 in a range from 1 to 1024.

Window 3 is displayed only if 'Other' has been selected as isotope 1 or isotope 2 in dual label counting. Enter the limits for window 3 in a range from 1 to 1024 or 0 - 0 if no third window is used.

Use mask adapter - if you select Y for this then the mask adapter will be used for the counting. Note: this must be set to Y if you want to use the injector because the injector uses the mask adapter. If you select N the parameters lines following as far as parameter 12 will not appear.

Use injector - select Y if you actually want to use the injector system. If you select N then even though the mask adapter with injectors is in position, no injection will be done. In the latter case lines from this one as far as parameter 12 will not appear.

Dispensing - you should select automatic dispensing. The other option, Manual is not available at present.

Dispensing - you can select the dispensing speed to be high, low or a custom speed set in the system settings.

Dispensing modules - here you give the number of modules to be used. If you have a single detector instrument you can have up to four injector modules. If you have 2, 3 or 6 detectors you can have one or two modules. In order specify the modules used use the following codes:

One module or only the first module	= 1
The second of two modules	= 10
Both of two modules	= 11
The third of three modules	= 100
The first and third of three	= 101
etc up to	
All four of four modules	= 1111

6. Isotope 2

Displayed only in dual label counting. The higher energy isotope is selected. The alternatives are the same as for parameter 5 with ^{14}C as default isotope.

7 Sample volume

Displayed only if Use injector has been selected to be Y. This parameter defines the original amount of sample in the well before dispensing. This is a mandatory entry and it is used for checking that the dispensed volumes do not exceed the maximum allowed volume. The value is an integer number in micro litres (µL). The minimum value is 0. The default value for this entry field is empty.

8. Dispensing volume

Displayed only if Use injector has been selected to be Y. This parameter defines the amount of liquid to be dispensed. The value is an integer number in microlitres (µL). The minimum value is 10 µL. The default value is 10 µL. There is a separate parameter for each module used. In a single detector counter this can be up to 4 modules and in multiple detector counters, up to 2 modules.

9. Delay time after dispensing

Displayed only if Use injector has been selected to be Y. The Delay Time defines the time to wait after dispensing, before the counting starts. There is a separate parameter for each module used. The resolution is 0.1 seconds. The default value is 0.1 second.

10. Counting time

The counting time is given in seconds. The minimum value is 0.1 and the maximum is 999 999.9 seconds. The default value is 60.0. If Use injector was selected for paramer 5 then there will be as many counting time parameters as there are dispensing modules.

12. Precision (2 sigma)

Counting stops when the standard deviation is below the two sigma value in all detectors. Precision is given in percent in a range from 0 to 99.9. 0 means that no precision limit is used. The default value is 0.2 %.

20. Count all positions in cassette

Press Y (Yes) if you want to count the whole cassette (default) or N (No) if you want to specify the positions to be counted. If No has been selected the next parameter will appear:

21. Cassette type

Type 1 if you use cassettes with 96 wells (default) or 2 if the cassettes have 24 or 384 wells.

22. Active rows

If you answered No on line 20 you will have to specify the rows to be counted for the first cassette by typing the letters for these rows. E.g. 'AC' means that samples A1 to A12 and C1 to C12 will be counted. For a more detailed specification, i.e. giving the positions instead of rows only, press R (Row coding). In this case specify which positions are to be counted for each row. The positions are in order from 1 to 12 (or 6 for 24-well plates). Type 1 to count a position or 0 to skip it. E.g. Row A: ->0011110 means that positions A3, A4, A5 and A6 should be counted. You do not have to enter all the zeros at the end of the line; the row end is filled with 0 or 1 according to the last typed character. See the following example:

```
20 Count all positions in cassette (Y/N)        Y ->N
21 Cassette type:  1)96  2)24                   1 ->
22      Cassette 1:(96)     Rows:              AB ->R
                              Row A: 111111111111 ->0011110
                              Row B: 001111000000 ->0000110
                              Row C: 000011000000 ->0
                              Row D: 000000000000
        Cassette 2:(96)     Rows:         ABCDEFGH ->AB
        Cassette 3:(96)     Rows:         ABCDEFGH ->/
```

An alternative method is to type the column numbers e.g. 5,6,7 equals 000011100000, 1, equals 100000000000 and 3,, equals 001111111111.

After pressing Enter you will be asked for positions for the next row. The default values are the same as you have given on the previous row.

Press / when you want to stop specifying counting positions. The rest of the rows will then be counted as the last edited row. In the example above, the following positions will be counted for cassette 1: A3, A4, A5, A6, B5 and B6. The following cassettes can then be coded in the same way as the first one. Press / once again to exit cassette coding. If there are more cassettes

in a batch than there are defined cassettes, the rest of them are counted according to the last cassette specification.

W can be pressed to code additional cassettes later.

X can be pressed to change the cassette type (96 to 24 (or 96 to 384) and vice versa) for cassettes 2 to 16, see the example:

```
20 Count all positions in cassette (Y/N)        Y ->N
21 Cassette type:  1)96  2)24  3)12             1 ->
22      Cassette 1:(96)     Rows:         ABCDEFGH ->R
                              Row A: 111111111111 ->3,4,5,6
                              Row B: 001111000000 ->5,6
                              Row C: 000011000000 ->0
                              Row D: 000000000000
        Cassette 2:(96)     Rows:         ABCDEFGH ->X
        Cassette 2:(24)     Rows:             ABCD ->AB
        Cassette 3:(24)     Rows:             ABCD ->/
```

23 Plate orientation

Select 2 if rotated plates (A1 at the bottom right corner) are used, 3 if mirrored (A1 at the top right corner), and 4 if both rotated and mirrored (A1 at the bottom left corner).

30. Printer output

There are four printer output alternatives:

1) No
2) Short
3) Long (default)
4) Programmable

1) No
If No is selected no results are printed.

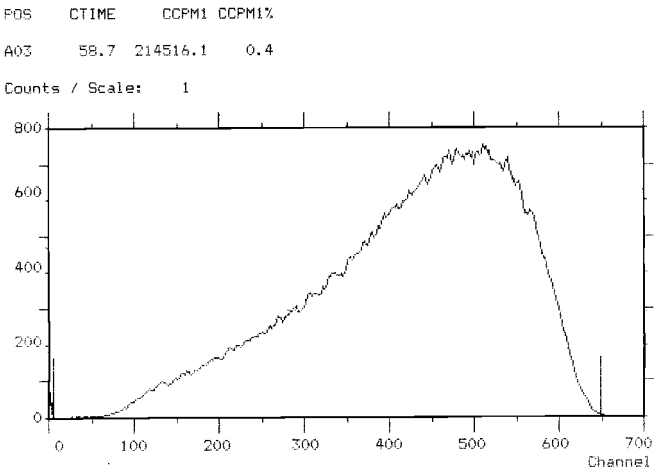
2) Short
If Short is selected, sample results are printed using the short results format: `POS, CCPM1, CCPM1%`. In dual label `CCPM2` and `CCPM2%` are added. `CCPM` is replaced with `DPM` in DPM counting. In luminescence counting it is `POS, LCPS` and `LCPS%`.

3) Long
The long output contains: `PROT, POS, CTIME, SQP(I)` (or `AQP(I)`), `CCPM1, CCPM1%`, plus `CCPM2, CCPM2%` in dual label counting. In DPM counting `DPM1` and `DPM1%` are added. In luminescence counting the output is `PROT, STIME, POS, CTIME, CPS1, LCPS, LCPS%`.

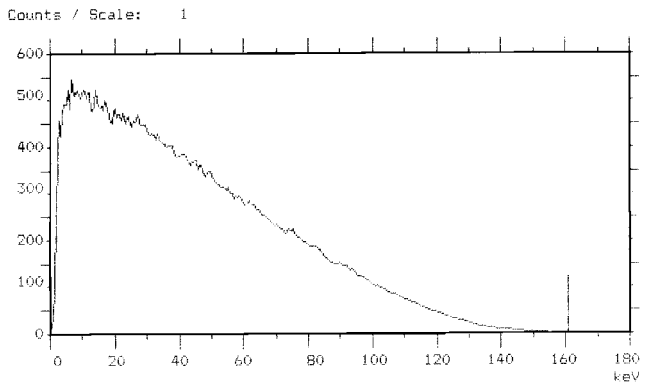
4) Programmable
The output items and arithmetic expressions are defined separately on the next line. Sample quality monitors can also be selected in the programmable output. Press ? or H to get a list of output items, monitors and rules for using own formulae. See also chapters 2.1 'Beginning operation', 2J.23 'Results', 2.29 'Statistics' and 2.21 'Monitors and flags'.

`If SPECTRA` is selected, three more lines are shown:

The first asks for the energy scale to be selected. It can be logarithmic with the units in channels or linear with the units in keV or both of these. Next you are asked to specify the range of the plot. This will be channels or keV depending on your previous selection. Finally you are asked for the spectrum size, small, medium or large. A smaller size will give faster output. The two figures following illustrate the type of plots you may get.



Spectrum with logarithmic scale



Spectrum with linear scale

32. Display output

This output is selected when you want to display results on the screen. It can be chosen independently of the printer output.

Display output has the same output alternatives as the printer output.

The default programmable output, `PRINTER` means the same data as specified for printer output.

34. External output

This output is selected when sending data to an external device such as a mainframe computer connected to the MicroBeta via port 2. The data is in ASCII format (text files).

The output alternatives are the same as in printer and display output, except that alternative 1, No output, is the default value.

The default programmable output, `PRINTER` means that the same data is specified as for printer output.

36. File output

The counting results can be stored on a floppy disk or hard disk on the PC or MicroBeta, or on the server of a local area network (LAN) (section 2.8 Datafiles). The default setting is 1, no file output. The alternatives are the same as in printer output.

The default programmable output, `PRINTER` means that the same data is specified as for printer output.

The result files are named so that the first part of the name (the part before the period) is the protocol number and the second part (after the period) is a running number indicating the assay's execution order. E.g. the first file for protocol 5 is named 5.001, the second 5.002, etc.

Result file names for normalizations begin with N (e.g. N1.001) and standardizations with an S (e.g. S1.001).

The disk drive (A:, B: for floppy disks, C: and D: for hard disks, F: etc. for LAN drives) and the path (e.g. `\MB\DATA\`) for the result files are chosen after the File output. This over-rides any path set in System mode. The drive and path defined in the System state is the default value (see chapter 2J.30.9 System/Data drive).

This is the terminal PC path. If results should be stored on the MicroBeta disk, then the path must begin with an @, e.g. `@B:\DATA\`.

If a VT52 or VT100 terminal is used then results are stored on the protocol disk as default (directory `B:\RESULTS`), see 2.30.10 Terminal.

The file name extension (the part after the period) can be changed on the next line. This must be a number in the range 1 to 999.

Note: this line is not available if UltroTerm is used.

40. Change special features

Press Y to change special features. These are counting control, background, half-life and chemiluminescence correction, password and special plate type.

41. Counting control

This and the following questions will be displayed only if Y has been selected on the previous line.

Press Y to change the counting control parameters. These are number of repeats, replicates and cycles.

If N is selected then these parameters have no effect.

The number of repeats is the number of times each sample will be counted. The minimum value is 1 (default) and the maximum is 999. If repeat is greater than 5, results are not sorted and statistical results are not printed. Repeats cannot be selected in crosstalk.

The number of replicates specifies how many replicates there are for a sample. This is used for evaluating sample preparation errors. The minimum value is 1 (default) and the maximum is 99.

The number of cycles is the number of times a batch of sample cassettes will be counted. The minimum is 1 (default) and the maximum is 99. If the number of cycles is greater that 1 then you will be asked to give the Cycle delay. This is in minutes and is the time between when one cycle ends and the next one begins.

See chapter 2J.6 Counting control for more information.

42. Background correction

Press Y to use background correction. You can choose between using the background measured in normalization, giving the position for a background sample or giving a value to be subtracted from the sample CPM. Answer Yes to the background sample question if you want to use a background sample. Enter the position for this sample. H12 (96-well, 1-6 detectors), G12 (12-detectors), D6 (24-well) or P24 (384-well, 1-6 detectors) is the default. Enter also the counting time (60s is the default). Note that the background sample must be in a position reached by every detector (see chapter 2.9 Detectors for these positions). The measured backgrounds are subtracted from CPMs when calculating CCPMs.

Answer No to the background sample question if you want to give a background value. The value, given in CPM, is subtracted from the sample CPM for an isotope. Two values are given, one for each window, if dual label counting is used. In dual label DPM counting, three values are given. The value is in the range from 0.0 (default) to 99.9 CPM or 999 999.9 LCPS.

Note: in the normalization protocol press Y if you want to count the background plate.

43. Half-life correction

Press Y if you want to use half-life correction. The half-life for the isotope is displayed, e.g. 107 500 hours for ^{3}H. No half-life value is given for ^{14}C because it is so long. Note that you must always specify the half-life if you have selected an isotope of type 'Other'. It must be in a range from 0 (no half-life) to 999 999.99 hours (114 years).

If normalization or standardization protocol is selected on line 3 then half-life is as specified in this protocol and it cannot be changed in the counting protocol

Press Y on the next line if you want to set zero time, otherwise the activity is corrected to the time when counting of the first sample of the assay was started.

The zero time consists of date and time. First enter the date in the format `DD-MMM-YYYY`, where `DD` is the day number (1 - 31), `MMM` the month in letters (`JAN, FEB, MAR, APR, MAY, JUN, JUL, AUG, SEP, OCT, NOV and DEC`) and `YYYY` the year, e.g. `15-JUN-2000`. The date can also be in the format `YYYY-MM-DD`, e.g. `2000-06-15`. Then enter clock time in the format `HH:MM:SS`, where `HH` is hours in the 24 hour mode (0 - 23), `MM` minutes (0 - 59) and `SS` seconds (0 - 59), e.g. `14:05:30`. It is not necessary to give seconds. The default value for zero time is the current time. See also chapter 2.14 Half-life.

44. Chemiluminescence correction

Press Y to use chemiluminescence correction, otherwise N (default). This correction is done with a correction formula, see chapter 3.3 Calculation methods.

45. Password

Press Y to select a password if you want to prevent other users from changing your protocol. Enter the password, maximum 12 characters. This will NOT be echoed to the screen. You will also be asked to retype it to prevent typing errors. The protocol cannot later be edited or deleted without knowing the password. You must therefore try to remember it. Only the MicroBeta supervisor has the authority to list the passwords (see chapter 2J.30 System/Passwords). He or she can help you if you have forgotten your password.

46. Special plate

Select Special plate type. Wallac is the standard plate. Betaplate filtermat plates and other 24-well plates have different dimensions and are counted using just one detector. However, it is possible to count other 24-well plates with all detectors. This selection comes after the plate dimension questions. For Betaplate filtermats you can select the 96-well plate format output. This is a composite of four 24-well plate format outputs. If you select the Other option for plate type, you must give the plate dimensions. These are the distances of the centres of the A1, A6 and D1 wells from the plate sides. Special cassettes must be used for Betaplate filtermat plates and other 24-well plates. The default dimensions 17.3/13.4/113.6/71.5 are for the Costar plate type. For the Falcon plate type the dimensions 14.5/13.4/110.7/71.5 should be used.

The default dimensions for a special 384-well plate are 11.8/9.0/115.3/76.5. These are the distances of the centres of the A1, A24 and P1 wells from the plate sides.

Note: this line is not available if 96-well cassette has been selected elsewhere in the protocol.

47. Autoquench correction

Automatic quench correction can be done in single label CPM counting. Isotopes must be 1, 2, 3, 4 or 6, and a non-crosstalk CPM normalization protocol must be selected.

48 Delay between plates

The delay between the end of plate measurement and the start of the next plate measurement can be specified. The range is 0 to 9999 minutes.

2J.25.8 Parameters in a normalization protocol

The parameters are much the same as in the counting protocol with some exceptions. Information whether normalization/standardization has been done is given after the protocol name. The following counting protocol parameters are not included in the normalization protocol:

```
2.  Counting mode
3.  CPM normalization/DPM standardization protocol
20. Count all positions in cassette
21. Cassette type
41. Counting control
```

The following parameters are new or changed when compared with the ones in the counting protocol:

3. Crosstalk correction

Press Y to use crosstalk correction otherwise select N. see chapter 2.7 Crosstalk correction for further information.

10. Counting time for background

The counting time for the background and crosstalk counting samples in crosstalk counting is given in seconds. The minimum value is 0.1 and the maximum is 999 999.9 seconds. The default value is 60.0.

11. Counting time for standards

The counting time for the standard samples is also given in seconds, from 1 to 999 999.9 with 60.0s as the default value.

41. Isotope activity setting

Press Y to set activities for normalization samples. This is isotope dependent and given in DPM. The range is from 1000.0 to 999 999.9. The default value is 200 000 for isotope 1 and 100 000 for isotope 2.

If activities are given, absolute normalization is done with efficiencies as count rates divided by the given activity.

If N is selected, relative normalization is done with efficiencies as a fraction of the highest detector count rate.

47. Strip plate

Press Y if you use plates where crosstalk is different in the X and Y directions. The crosstalk sample in G10 is counted in this case.

See chapter 2J.23 Normalization for further information.

2J.25.9 Parameters in a standardization protocol

The parameters are much the same as in the counting and normalization protocols with some exceptions. JET features are not available with standardization but the mask adapter can still be used. Information whether normalization/standardization has been done is given after the protocol name. The following counting protocol parameters are not included in the standardization protocol:

```
2.  Counting mode
3.  CPM normalization/DPM standardization protocol
20. Count all positions in cassette
21. Cassette type
41. Counting control
42. Background correction
```

The following parameters are new or changed when compared with the counting protocol:

2. Sample type

Select normal or scintillation proximity assay for sample type. ParaLux counting is set automatically if SPA is selected.

3. Crosstalk correction

Press Y to use crosstalk correction otherwise select N. See chapter 2.7 Crosstalk correction for further information.

14. Easy DPM

Press Y to use Easy DPM quench curves. Only the two DPM normalization samples are counted in standardization.

20. Number of standards

Give the number of DPM standards used for standardization. The value must be in a range from 2 to 12 (6 in dual label) with 6 as default.

21. Isotope 1 activity

Give the isotope activity in DPM. The range is from 1000.0 to 999 999.9 with 200 000 as the default value.

23. Isotope 2 activity

Give the Isotope 2 activity in DPM. The range is from 1000.0 to 999 999.9 with 200 000 as the default value.

24. Standard curve fit selection

Press Y to use standard curve fitting, otherwise N. If No is selected, smoothing spline with automatic smoothing will be used or linear interpolation if SPA is selected for the sample type.

25. Curve fit method

This line is displayed only if you have selected Yes on the previous line. The methods for standard curve fitting are:

1) Smoothing spline (default)
2) Interpolation spline
3) Linear interpolation (default for SPA)
4) Linear regression

Select a method by typing the number and Enter. If you have selected the first alternative, smoothing spline, you will be asked if the method is to include automatic smoothing. Press Y (default) if so. Otherwise press N and you will be asked for a smoothing parameter. Enter a value between 0.0 (no smoothing) and 1000.0 (maximum smoothing), with 1.0 as default.

26. Edit standard curve

Press Y to edit the quench parameter, efficiency and weight values for a standard curve or to delete curve points.

41. Adjusted activities

Press Y if you want to adjust the activities for standards, otherwise the activity given on line 21 will be used for every standard. After pressing Y you will be able to change activities for the same number of DPM standards as given on line 20. You can also change the activities for the two Easy DPM standards. The range is from 1000.0 to 999 999.9 DPM. The value given on line 21 is default. See chapter 2.12 DPM Standardization for further information.

2J.27 Robotic loading interface

The robotic loading interface is a special door/adapter system that brings a cassette outside so that a robotic arm can change the plate.

In the MicroBeta program the robotic loading interface is selected as follows:

```
(S)ystem
  (L)evel
    (T)esting
      Detector/shelf (u)sage
        Use shelves: 1)1 2)16 3)32 ->1
  (L)evel
(N)ormal counting
```

The operation is controlled using the RS-232C port 2 (external port) of MicroBeta. When the cassette is outside, MicroBeta sends the text Ready and carriage return / line feed. The possible commands are:

H or ? - list commands
Nxx - start normalization using protocol number xx
Dxx - start standardization using prot. number xx
xx - start counting using protocol number xx
A - start counting using IDs.

All Ready commands must be followed by a carriage return / line feed or #. Counting can be interrupted with O. After a plate has been measured and the cassette is outside, MicroBeta sends the text Continue and carriage return / line feed. At this point O stops counting and any other character continues counting.

If plates are counted in the inverted position (A1 at the bottom right corner) then Plate orientation "Rotated" should be selected in the counting protocol. This selection reorders results so that outputs POS and SEQ are correct.

Warning

The mask adapter has to be moved manually into the counting chamber before starting counting.

2J.30 System

2J.30.1 Selecting System

System settings control the general way MicroBeta works - its defaults, hardware connections etc. To enter the System state press S in the Ready state. You are then given a number of options as example 1 shows. These are described in more detail here.

Example 1

```
(H)elp   (I)nfo
(C)ount  (P)rotocols  (S)ystem

Ready>S

(H)elp        (Q)uit            MS-D(O)S
(E)rrorbeep   (S)tatus display  (V)ersion
(C)lock       (R)S-232C         (P)rinter
(D)ata drive  Ter(m)inal        P(a)sswords
Customi(z)ing (I)jector module

System>
```

2J.30.2 (H)elp

Press H to get help about the various selections.

2J.30.3 (E)rror beep

User or instrument error normally causes a beep. This can be switched off by selecting N.

Example 2

```
System>E

Error beep (Y/N) Y ->N
```

2J.30.4 (S)tatus display

Normally when counting is occurring its status is shown on the Terminal PC screen (see chapter 2.5 Counting for more details). The counting status display can be switched off or only currently counted positions can be shown.

Example 3

```
System>S

Status display:  1)No   2)Current positions
                 3)Whole plate                      3->
```

2J.30.5 (V)ersion

Show the program and MS-DOS version numbers.

Example 4

```
System>V

MicroBeta program V5.0. Copyright (C)
Wallac Oy, 1998. All rights reserved.

MS-DOS V3.2. Copyright (C) Microsoft
Corporation, 1987. All rights reserved.
```

2J.30.6 (C)lock

This allows the date and time to be set. MicroBeta has a calendar clock with battery back-up so you do not have to set it each time the power has been switched off, normally only when the instrument is installed. Details of setting the clock are given in chapter 2.3 Clock. When you set the clock the Terminal PC date and time are also set if GenTerm is used.

Example 5

```
System>C

Date 12-Jul-2000 -> 13-Jul-2000
Time       14:44 -> 9:30

Set clock(Y/N)->Y
```

2J.30.7 (R)S-232C

There are three RS-232C communication ports which can be used:

1 for the Terminal PC
2 for an external computer
3 for injector module communication

You can specify the communication parameters for each of these ports separately. Give the number of the port and press Enter.

The first parameter that can be changed is the speed of transferring the data, the Baud rate. The possibilities are: 300, 600, 1200, 2400, 4800 or 9600.

The default value for the terminal and external ports is 9600. For the printout port it is 4800. If these values are not suitable then select the ones you need.

Next is the parity selection, you can have None, Odd or Even where None is the default.

The number of data bits can be 8 or 7 where 8 is the default.

The number of stop bits can be 2 or 1 where 2 is the default.

Lastly you can select the kind of checking process for the data transfer, the handshaking. It can be None, DTR or XON/XOFF where the default is DTR.

Confirm the selection of these parameters by typing Y twice. When you have done this the program prints the original and the new settings and returns to the System state if serial port 2 or 3 setting is in question. However when changing the terminal port (port 1) setting the program then shows a help text that informs you to set your terminal to the same settings which were selected for MicroBeta before confirming the new setting. When ready press any key. The program should then ask:

```
Do you see this text (Y/N)?
```

If the text appears it is quite sure that the communication works and you can press Y to confirm the change of terminal port setting. If the text does not appear it is obvious that your new terminal and MicroBeta serial port settings differ. In that case check from the printer the new MicroBeta setting and modify your terminal setting if required until you see the above text. If you cannot make the communication work you have still one possibility. Set the terminal to the original serial port setting. Switch off the counter and switch on again. Restarting MicroBeta at this phase returns the original terminal port communication setting. Note: You can lose communication with the counter also by changing the serial port setting of your terminal alone. Usually then some strange characters appear around the screen and commands do not function. To get communication functioning again you can change terminal settings in the following way if you do not remember the terminal port setting of MicroBeta:

- Try different baud rates. Press e.g. space bar after setting each baud rate.

- If there is no success then change the number of data bits (usually 7 or 8 bits come into question) and try different baud rates again.

- Usually parity and handshake differences should not prevent normal manual terminal use.

- Another method is to switch the counter off and on again.

The correct parameters (terminal type, baud rate and data bits) are set automatically after instrument restart. If they differ from the current parameters then a text like the following is shown: `Incorrect terminal baud rate. 9600 bps used. Please set terminal RS-232C parameters in (S)ystem /(R)S-232C`.

When it seems that communication is working, go to the MicroBeta System-mode and check the serial port 1 settings and make sure that they are identical with settings of the terminal.

Example 6

```
System>R
RS-232C port number                  1->
Baud rate:  1)300  2)600  3)1200
            4)2400 5)4800 6)9600     6->
Parity:  1)None  2)Odd  3)Even       1->
Data bits                            8->
Stop bits                            2->
Handshake:  1)None  2)DTR  3)XON/XOFF 2->
Set terminal port parameters      (Y/N)->Y
Are you sure?                     (Y/N)->Y
Do you see this text (Y/N)?Y
```

Note: for serial port 3, the connection to the injector modules, the parameters must be: Baud rate = 6, Parity = 1, Data bits = 8, Stop bits =2 and handshake = 1.

2J.30.8 (P)rinter

Set the printer type and the number of characters per inch. The options are: IBM or Epson FX and the number of characters per inch 10, 12 or 17. The defaults are Epson FX and 12 cpi.

Example 7

```
System>P

Printer type:    1)IBM  2)EpsonFX           2->
Printer charac.per inch:  1)10  2)12  3)17  2->
```

2J.30.9 (D)ata drive

Select diskette or hard disk drive of the terminal PC or MicroBeta on which the results are to be stored (when using UltroTerm define the result file path in UltroTerm Result filing options). The result file path can also be given in each protocol.

Example 8

```
System>D

Data drive:  1)A:  2)B:  3)C:  4)Path   4->
Path name:                 C:\MB\RESULTS\->
```

The options are:
1 = diskette drive A. Select this if you have:
(a) a single drive terminal PC (no other possibilities)
or
(b) a hard disk PC but you want to store results on the diskette (e.g. to be transferred somewhere else).
2 = diskette drive B. Select this if you have a dual diskette drive terminal PC. Then terminal emulator software can be kept in drive A all the time (e.g. to enable automatic restart after power failure).

3 = hard disk drive C. Select this if your terminal PC is equipped with hard disk and you want large storage capacity. If the GenTerm terminal emulator is used, then results will be stored into the directory which was active when GenTerm was started.

4 = full disk drive and directory path. Use this if options 1-3 do not match your requirements. E.g. if you want to store results:
(a) on a hard disk drive other than C (e.g. D),
(b) on a local area network (LAN) connected to the terminal PC,
(c) in a specific directory
or
(d) on the MicroBeta drive.

If 4 is selected then the program asks for the full drive and directory path. Here understanding of MS-DOS drive and directory concepts is needed. The directory path given here will be used as such without any checking and modifications in front of the file name. Note that the directories must exist before any data can be sent to them. Use the command MKDIR in DOS to make new directories.

Examples (referring to examples a-d above):

(a) Path name: D: ->
- Text D: will be inserted in front of result file name. E.g. D:2.003 , the file will be stored in the active directory on drive D.

(b) Path name: F: ->
- Here it is supposed that drive F: belongs to the LAN server. Text F: will be inserted in front of the result file name. E.g. F:17.002 , the file will be stored in the active directory of the LAN server drive F.

(c) Path name: `C:\MB\RESULTS\ ->`
- Text `C:\MB\RESULTS\` will be inserted in front of result file name. Note that if you specify an exact directory path for the result files then the path name must end with backslash \. For example the path `C:\MB\RESULTS\3.023` stores result file 3.023 into the directory `\MB\RESULTS\` of hard disk drive C.

(d) Path name: `@B:\DATA\->`
- Results are stored on the MicroBeta protocol disk B in directory `DATA`. The character @ at the head of the path shows that the MicroBeta disk is to be used for storing the results.

2J.30.10 Ter(m)inal

Select the terminal emulation (see chapter 2.31 Terminal emulators for details) that your terminal or terminal PC uses. The additional questions depend on the emulation selected. The possible emulations are:

1 = GenTerm in WT emulation mode. This emulation is fully tailored to be used with MicroBeta, so the only things the user has to specify are (see example 9):

Example 9

```
System>M

Terminal emulation:
1)GenTerm  2)UltroTerm  3)MultiCalc
4)VT52     5)VT100      6)MBW              2->1
Number of lines on screen                 24->
Print through terminal (Y/N)               N->
Do you accept the new terminal setting? (Y/N)->N
```

- the number of lines on the screen.
- whether the printer should be connected to the terminal PC or not (see 2J.30.10.5).

2 = UltroTerm (see example 10). Select this if you are using the UltroTerm terminal emulator. UltroTerm V2.0 or later is required.

Example 10

```
System>M

Terminal emulation:
1)GenTerm   2)UltroTerm   3)MultiCalc
4)VT52      5)VT100       6)MBW             2->
Number of lines on screen                  23->
Print through terminal (Y/N)                N->
Do you accept the new terminal setting? (Y/N)->Y
Prepare your terminal ready for emulation change.
```

```
Press any key when ready.
```

3= MultiCalc VT52. Extensions to enable result file saving to PC disk when using MultiCalc.

4 = VT52 (see example 11). Select this if you are using an emulator that can provide only this emulation.

5 = VT100. This emulation is quite popular in commercial terminal emulation programs. If your terminal emulator implements both VT52 and VT100, select VT100 because it supports a better range of MicroBeta features i.e. counting results displaying (screen attributes, result window scrolling etc.).

6 = MBW (MicroBeta Windows workstation VT52). If this is selected then results sorting and live data automatic sending must be specified. The workstation program sets the terminal and other settings automatically.

Additional questions:

2J.30.10.1 Number of lines on screen

Possible values from 15 to 25, the default value is 24 lines. Some terminal emulator programs can scroll the whole screen if something is written at the lower right corner of screen (e.g. column 80 on line 24) which mixes the status display. If you encounter this problem give here a smaller value (e.g. 23 lines). You can select a smaller value also if your terminal PC cannot display 24 lines (e.g. some portable PCs can display 16 lines).

2J.30.10.2 Block graphics

For VT52 and VT100, default Y=Yes. If your terminal shows some strange characters around the status display and it seems that it cannot display block graphic characters, select N=No.

2J.30.10.3 Send result files to terminal

For VT52 and VT100. The method of sending result files to the terminal PC depends on the terminal emulator software. Often a terminal emulator can capture data between a start string and a stop string. If your terminal emulator is capable of doing this then answer Y=Yes. Then MicroBeta stores a result file at first on the protocol diskette and sends it to the terminal as one block as:

```
Start of assay:XX
... ... Result data
...
End of assay
```

where xx is the protocol number.

If your terminal emulator cannot capture data then answer N=No. Then the result files are stored on the protocol diskette of MicroBeta but not transferred to the terminal PC.
Note 1: Result file will be output if protocol line 36 FILE OUTPUT is set to other than 1 (No output).

Note 2: See chapter Results for details.

2J.30.10.4 Automatic result file deletion

For VT52 and VT100, default Y=Yes. The capacity of the protocol diskette is limited (300KB - 1.3MB depending on the number of protocols) and it is capable of storing the results from at least 20 cassettes if the output selection for `FILE OUTPUT` does not specify too many different printout fields. If this selection is set to Y=Yes then the result files on protocol diskette will be deleted automatically when starting counting and after sending a result file to the terminal PC is complete. If you want to ensure that your data has been transferred to another diskette or to some PC then answer N=No. In that case after starting counting the program confirms deletion before operation by asking:

```
Delete result files saved on protocol disk?
```

and you can answer N=No to reject deletion or Y=Yes to accept deletion.

2J.30.10.5 Print through terminal

Select here where to connect the printer. Answer: N=No if you do not need to connect the printer to the terminal PC. Connect instead the printer to serial port 3 of MicroBeta with serial cable 1221-502.

Y=Yes to connect the printer to the terminal PC. In this case connect the printer to the parallel port of the terminal PC with a Centronics cable 1221-122.

2J.30.10.6 Exit terminal setting

To exit terminal setting press slash / and Enter on any line. Otherwise, after you have answered all the questions described above, the program confirms that you accept the change. If not, press N, or else press Y which causes the following message to appear:

```
Prepare your terminal ready for emulation change. Press any key when ready.
```

Now you can make the preparations possibly required for the emulation change (e.g. to switch from GenTerm to UltroTerm). When ready press any key. Then if the change succeeded the program returns to the System state. However if the message `Terminal type check failed!`

appears (see example 11) it means that MicroBeta has found that the terminal program does not use the same emulation as was selected (MicroBeta sends an identity inquiry to the terminal and the answer sent by the terminal emulator software is different for each emulation - GenTerm, UltroTerm, VT52, VT100). Answer N if you want to cancel the new terminal emulation. However if you know that your terminal has the correct emulation or you want to switch to the new emulation later you can answer Y.

Example 11

```
System>M

Terminal emulation:
   1)GenTerm 2)UltroTerm 3)MultiCalc
   4)VT52    5)VT100     6)MBW              2->4
Number of lines on screen                  24->
Block graphics (Y/N)                        Y->
Send results file to terminal (Y/N)         N->
Automatic result file deletion (Y/N)        Y->
Print through terminal (Y/N)                N->
Do you accept new terminal setting? (Y/N)    ->Y
Prepare your terminal for emulation change.
Press any key when ready
Terminal type check failed!
Do you accept the new terminal setting? (Y/N)->N
```

2J.30.11 P(a)sswords

Press A to print protocol passwords, entered on line 45 in the protocol editor (see chapter 2J.25 Protocols). This is possible only if no System password is in use or in Setup mode (see 2.30.15).

Example 12

```
System>A

Printing protocol passwords, press / to exit
```

2J.30.12 Customi(z)ing

Press Z to set environment strings to customize the program. The environment string name and value must be given. For a list of possible strings, see (I)nfo/Customi(z)ing. Giving D as the value sets the default value for the string.

Example 13

```
System>Z

Customized: MENU = N
Environment string name         -> MENU
```

```
MENU                            -> D
Environment string name         -> /
```

2J.30.13 MS-D(O)S

Press O to get to the MicroBeta MS-DOS. You must confirm this twice as example 14 shows, after which the text `MicroBeta A:\>` appears:

Example 14

```
System>O

Exit to MS-DOS? (Y/N) ->Y
Are you sure? (Y/N) ->Y

Type MB to start the program again

MicroBeta A:\>MB
(H)elp    (I)nfo
(C)ount   (P)rotocols   (S)ystem

Ready>
```

Type MB and press Enter to get back to the MicroBeta program.

Note: Avoid going to MS-DOS in routine work. The main reasons to go there are to make a backup copy of the protocol or program diskette.

2J.30.14 (I)njector

Press I to set injector parameters for MicroBeta JET.

Example 15

```
System>I

Mask adapter (Y/N)                                          Y->
Injector module (Y/N)                                       Y->
Raise detector (Y/N)                                        Y->
Multichannel pump (Y/N)                                     Y->
Number of channels                                          6->
Number of injectors                                         2->
Injector 1:
Syringe volume: 1)100uL 2)250uL 3)500uL                     3->
Aspirating tube I.D. ["]                                0.053->
Aspirating tube length ["]                               27.0->
Dispensing tube I.D. ["]                                0.030->
Dispensing offset [uL]                                      0->
Injector2:
Syringe volume: 1)100uL 2)250uL 3)500uL                     3->
Aspirating tube I.D. ["]                                0.053->
Aspirating tube length ["]                               27.0->
Dispensing tube I.D. ["]                                0.030->
Dispensing offset [uL]                                      0->
Dispense fast command string  S10L7IAxOv1000V4000c2700L20A0R ->
Dispense slow command string  S10L7IAxOv1000V2000c2700L20A0R ->
Dispense custom command string S10L7IAxv1000V3000c2700L20A0R ->
Wash command string                          S10IAxV3800OA0R ->
Tip init command string                    S10L7OM200A0A24IR ->
Prime command string                         S10IAxOV3800A0R ->
Suck-back command string                     S10OAxIV3800A0R ->
Initialize command string                              Z6R ->
Stop command string                                      T ->
Dispensing volume max [uL]                             250 ->
Store data? (Y/N)                                        ->
```

2J.30.14.1 Mask adapter.

This is the plate that fits between the detectors and the microtitration plate. The number of holes in the plate correspond to the number of detectors. This parameter allows you to select if the mask adapter is to be used. It must be used if you want to use the injector system since the injector tubing is fitted into the mask adapter.

2J.30.14.2 Injector module

This parameter must be Y if you want to use injection.

2J.30.14.3 Raise detector

This parameter must be Y if you want to use injection because the upper detector must be raised to make space for the mask adaptor and injector(s).

2J.30.14.4 Multichannel pump

There can be from 1 to 6 channels for the pump. If the pump is a single channel one, i.e. for a single detector MicroBeta JET then select N here. Otherwise select Y for a 2, 3 or 6 channel pump.

2J.30.14.5 Number of channels

Give here the number of channels 1, 2, 3 or 6.

2J.30.14.6 Number of injectors

A single channel system can have up to four injectors, whereas a multiple channel system can have a maximum of two injectors. Give the number for this parameter.

2J.30.14.7 Injector 1

As many injectors will appear as there are selected. Each injector has its own set of paramters as follows.

2J.30.14.8 Syringe volume

This is the amount of liquid a syringe takes in during one stroke. There are three possibilities 100, 250 or 500 μL. Select the one you want.

2J.30.14.9 Aspirating tube I.D.

The internal diameter of the tubing from the liquid reservoir to the syringe. The units are inches.

2J.30.14.10 Aspirating tube length

The length of the tubing from the liquid reservoir to the syringe. The units are inches.

2J.30.14.11 Dispensing tube I.D.

The internal diameter of the tubing from the syringe passing through the injector assembly to above the well.

2J.30.14.12 Dispensing offset

This is the offset volume for injector modules and it is set in steps of 1 μL. It is a volume that effects the injector dispensing volume. If the volume is selected from the protocol as 10 μL and the offset is 0, then the dispensed volume is 10 μL. If the offset is 2 μL then when the protocol selection is 10 μL the real dispensed volume is 12 μL. So if in the injector there is for some reason a systematic error in the dispensing volume it can be corrected with the offset.

The following parameters are common for all channels and should not be changed by the user without contact with a service person.

2J.30.14.13 Dispense fast command string

This defines how fast the dispensing occurs for fast dispensing.

2J.30.14.14 Dispense slow command string

This defines how fast the dispensing occurs for slow dispensing.

2J.30.14.15 Dispense custom command string

This defines how fast the dispensing occurs for the custom dispensing. This can be set to the speed you require if neither slow nor fast speeds are acceptable.

2J.30.14.16 Wash command string

This defines how the wash procedure occurs. Wash is used to clean the tubing after use.

2J.30.14.17 Tip init command string

This ensures that there are no drops on the end of the tubing.

2J.30.14.18 Prime command string

This fills the tubing with liquid before operation can begin.

2J.30.14.19 Suck-back command string

This command causes the liquid in the tubing to be returned to the reservoir.

2J.30.14.20 Initialize command string

This prepares the system for use and is the first operation before any other injection operation.

2J.30.14.21 Stop command string

The is the command to stop operation.

2J.30.14.22 Dispensing volume max.

This is the maximum volume that may be in a well. It is the sum of the sample volume and the dispensed volume. It ensures that the well is not overfilled.

2J.30.15 (Q)uit

Press Q to quit from the System state.

2J.30.16 Se(t)up mode

If a system password is used then the system mode menu is quite short (see example 16).

Example 16

```
(H)elp        (Q)uit            MS-D(O)S
(E)rrorbeep   (S)tatus display  (V)ersion
(C)lock       Se(t)up mode

Ready>T
Password ->
(H)elp        (Q)uit            MS-D(O)S
(E)rrorbeep   (S)tatus display  (V)ersion
(C)lock       (R)S-232C         (P)rinter
(D)ata drive  Ter(m)inal        P(a)sswords
Customi(z)ing (I)jector module

System>
```

All the functions that can affect routine use are hidden. The person responsible for the MicroBeta system can make the hidden functions visible if he or she knows the password. Press T to get into the setup mode. The program then asks for the password. Type the password and press Enter. The characters you type are not displayed on the screen. If you enter a wrong password the program displays the message:

```
Incorrect password
```

Otherwise the program shows the full System menu.

To set a system password, exit from the MicroBeta program to MS-DOS as described earlier. After the text `MicroBeta A:\>` appears, type `PASSWORD xxxx` and press Enter (xxxx means the new password). If you forget the password then set a new one.

Part 3 General description

3.1 Instrument description

3.1.1 Introduction

This section describes the hardware features of 1450 MicroBeta TriLux and MicroBeta JET.

3.1.2 Combined liquid scintillation and luminescence counter

With MicroBeta TriLux you can count beta or gamma emitting samples or glow luminescence samples. With MicroBeta JET you can in addition count flash luminescence samples in which reagent injection is required. You get the luxury of several powerful counting techniques combined in one counter.

3.1.3 Microtitration plate based operation

In MicroBeta samples are counted in a microtitration plate. Before counting, the instrument itself determines which type of plate is being used.

The plate is placed in a special cassette for counting which you can identify with barcoded labels. Load the cassette into the cassette rack behind the door with the plate upwards and the ID labels towards you, then close the door.

3.1.4 Cassette rack and transportation system

The cassette rack is located inside the door and consists of 16 (32) shelves. These are numbered 1 to 16 (32) from the bottom upwards. The maximum capacity is 1536 (3072) samples when using 96-well plates and 384 (768) samples when using 24-well plates and 6144 (12288) samples when using 384-well plates.

The cassette rack is in its middle position when being loaded. A motor moves the rack up or down one step at a time. Sensors are used for identifying shelf numbers and for checking if there are cassettes on the shelves and whether they are 24, 96 or 384-well type. A sensor will cut off the high voltage if the door is opened during counting in order to protect the detectors.

One cassette at a time is moved to the transportation system. This is an X-Y transfer mechanism which first moves it to the ID reader which reads the barcodes on the cassettes. Then it is moved to the first counting position. After a cassette has been counted, it is moved back to the rack. The elevator moves the cassette rack down one position and the next cassette is moved to the transportation system.

3.1.5 Coding

Code labels can be used to identify which protocol (set of counting parameters) is to be used for counting the samples in a cassette. These identification labels are printed with barcodes, and the reader is situated in front of the detector block. There are identification labels for identifying the protocol and the cassette number. There are also three special function codes: STOP, NORM and STD. When a cassette carries a STOP code, it is counted normally, and after it the counting stops. NORM and STD show that the cassette carries normalization or standardization samples respectively. See chapter 2.4 Coding the cassette for more details. You can also identify the plate itself with a barcode, see section 2.4.6 Plate ID reader.

3.1.6 Detectors

One, two, three, six or in the case of MicroBeta TriLux twelve pairs of photomultiplier tubes view the samples in the measuring position. The detectors are placed in a 1x1, 2x1, 1x3, 2x3 or 3x4 formation respectively, see chapter 2.9 Detectors for pictures of this formation. The tubes of one pair are situated on opposite sides of the counting block, one above and one below the block. Each pair operates in coincidence mode which greatly reduces single photon background events. The out-of-coincidence signal is also measured simultaneously.

When a cassette moves into the counting position the labels on the cassette are read by the barcode reader. Then 1, 2, 3, 6 or 12 samples on the cassette are counted simultaneously according to the protocol specified by the label on the cassette.

Due to the relatively small sample volume there is such a low background count that no extra lead shielding is needed, thus reducing the weight of the instrument.

To avoid ambient light affecting the photomultiplier tubes, the inside of the instrument is painted black and the door has a light seal along its edge.

To protect the detectors, the high voltage of the photomultiplier tubes is cut off when the door is open. A warning message is displayed if counting is attempted while the door is not properly closed. See chapter 2.16 Interrupt.

3.1.7 Glow luminescence assays

With MicroBeta TriLux, luminescence assays designed to be completed on a solid support e.g. nylon membrane, glass fibre or coated plate, and in solution can be directly quantified.

Due to the high signal, luminescence reactions can be measured using single PMTs.

Traditional microtitration plates are totally opaque (black or white). These plates are counted with the upper tube and a 1450-105 cassette is used. The filters are counted with the upper or lower tube and a 1450-104 filter cassette is used.

A special feature when using MicroBeta as a luminometer is the 1450-466 coloured sealing tape. The saturation of PMTs is prevented and crosstalk between adjacent positions is minimized by cutting the intensity of luminescence light with the 1450-466 coloured sealing tape.

The luminescence units used are LCPS: luminescence counts per second i.e. corrected CPS-values/100.

3.1.8 MicroBeta JET features

In addition to the MicroBeta TriLux features described above, MicroBeta JET makes it possible to measure flash luminescence, dual luminescence and do kinetic studies because it allows reagent addition and mixing by dispensing.

MicroBeta JET can be purchased without the injector assembly or injector modules and these can be added later in the field by the user when required. Also additional injector modules can be added.

An injector module is a pump with 1, 2, 3 or 6 syringes each feeding tubing that goes to the mask adapter which positions the tips of the tubes above the well(s). Each syringe and hence piece of tubing constitutes one channel. A single detector instrument can have up to four modules each with one channel. A multiple detector instrument can have up to two modules, each with as many channels as there are detectors in the instrument i.e. 2, 3 or 6.

Each module and channel can be switched on or off independently.

The use of at least two injectors for the same well means that dual luminescence assays can be performed.

If reagent injection is selected in any particular counting protocol, the mask adapter (injector assembly) is driven into position above the microplate. The injector tips which are embedded in the mask adapter are automatically positioned over the wells to allow injection under optimum conditions. The light resulting from the reaction passes through the fibre optic coupling in the injector assembly to the upper PMT.

The mask adapter can also be used without injection simply for masking. It reduces the signal to the upper detector by a factor of ten which can be helpful in cases of luminescence with very high signal.

3.1.9 Robotic loading interface

The robotic loading interface is a special door/adapter system that brings a cassette outside so that a robotic arm can change the plate. In this case only one shelf is used.

Note: the mask adapter has to be moved manually into the counting chamber before starting counting.

3.1.10 Microcomputer control

Operation of the instrument and calculation of the results is performed by a combination of a terminal PC running a Wallac terminal interface program or the MultiCalc immunoassay program, and the instrument itself.

The built-in computer is composed of a 16-bit Intel 80186 microprocessor with 1 MB RAM and 64 kB ROM. There are two microfloppy 3.5 disk drives; each disk has a capacity of 1.44 MB. The disks loaded onto these drives contain the program and the protocols.

The Terminal PC is a personal computer that is used to run the terminal emulation program or MultiCalc and can be used to collect counting data for further evaluation.

Output possibilities can be selected by means of the programmable RS-232C interface with three output ports. The uppermost port sends the output to the terminal, the second to an external computer and the third to a printer or in the case of MicroBeta JET to the injector modules.

Should any fault occur, the built-in service test routines allow the service engineer to quickly diagnose the cause of the fault and, due to the flexible arrangement of the electronics, easily change any components where necessary.

3.2 Routine maintenance

3.2.1 Cleaning the instrument

The cassette rack should be kept clean. The cassettes should be kept clean in order to prevent dirt from coming between the detector blocks, to minimise the background and maximise the light collection efficiency.

In MicroBeta JET the optical fibres of the mask adapter (injector assembly) must be kept clean. If necessary, wipe them with a moist cloth.

Tubing should be washed with water using the Wash command.

Note: refer also to the reagent manufacturer's instructions to see if there is additional information on cleaning tubing.

3.2.2 ID label

Check the labels on the ID support plates. Those in bad condition should be replaced by new ones to guarantee correct reading of the labels. When fixing ID labels on the support plate, ensure that the area where the label is to be fixed is clean, e.g. that there is no perspiration from your fingers on it.

3.2.3 Power supply fan

Check that the cooling fan in the power supply unit is working by listening for its sound.

3.2.4 Checking the instrument

To check the instrument, it is recommended that at least once a month you run counting protocol number 99, the GLP (Good Laboratory Practice) protocol. Use this protocol to count the normalization sample plate supplied with the instrument. The background, ^{3}H and ^{14}C samples are counted with all detectors so background level, CPM values and SQP(I) values can easily be compared.

Recommended limits:

^{3}H		^{14}C	
Eff%	Bkg	Eff%	Bkg
>45%	max 50 cpm	>91	max 60 cpm

If the values obtained by running the GLP protocol are outside the recommended limits, please contact your service support person.

3.3 Calculation methods

3.3.1 Count rate

Count rate i in counts per minute (headings CPM, CPM1, CPM2, CPM3) is calculated using the equation:

$$i = 60*c/t \quad (1)$$

where c is counts in the counting window and t is dead time corrected counting time (heading CTIME) in seconds.

In ParaLux high efficiency mode:

CPM1 = CPM_C + CPM_NC

where CPM_C is normal coincidence count rate and CPM_NC is non-coincidence count rate between discriminator channel and 1024.

Count rate α-sigma percentage error (headings CPM%, CPM1%, CPM2%, CPM3%) is calculated using the equation:

$$\alpha*\delta i/i = \alpha* 100 / \sqrt{c} \quad (2)$$

Above and in what follows amu can be either 1.0 or 2.0 .

3.3.2 Normalization

3.3.2.1 Detector efficiency normalization

Detector efficiency normalization corrects for differences in detector responses. Efficiencies and efficiency errors of each detector are calculated and stored for count rate correction. Background corrected count rate r is calculated using the equation

$$r = i - b \quad (3)$$

where i is from equation (1) and b is the normalization background count rate for the same detector and counting window. ParaLux high efficiency backgrounds are subtracted first.

Efficiencies e(mn) are calculated using the equation

$$e(mn) = r(mn) / j \quad (4)$$

where r(mn) is isotope n background and half-life corrected count rate in window m and j is the given normalization activity or the maximum background corrected count rate of isotope n in window 1 (single label) or in window 5-1024 (dual label) or the average count rate (luminescence).

Efficiency percentage error is calculated using the equation

$$\delta e(mn)/e(mn) =$$

$$\sqrt{\{[\delta i(mn)/r(mn)]^2 + [\delta b(mn)/r(mn)]^2 + [\delta j/j]^2\}} \quad (5)$$

If j is the given activity then $\delta j=0$.

3.3.3 Corrected count rate

Corrected count rate (headings CCPM, CCPM1, CCPM2) is calculated in three steps in the following order:

- Background correction

- Detector efficiency correction or crosstalk correction

- Half-life correction

The first two corrections also affect the theoretical error (headings CCPM%, CCPM1%, CCPM2%) of the corrected count rate.

3.3.3.1 Background correction

If background sample is specified and measured or background is given in the protocol then count rate i is corrected for background using equation (3).

$$r = i - b$$

ParaLux high efficiency backgrounds are subtracted first.

If background sample is measured then the count rate α-sigma error becomes:

$$\alpha*\delta r /r = \alpha\sqrt{[(\delta i)^2 + (\delta b)^2]} / r \quad (6)$$

3.3.3.2 Detector efficiency correction

In single label counting detector efficiency correction for count rate is calculated using the equation

$$R = r / e \quad (7)$$

where r is from equation (3) and e = e(11) from equation (4) for the same detector as in normalization.

Efficiency correction affects also the corrected count rate α-sigma error,

$$\alpha*\delta R /R = \alpha\sqrt{[(\delta r/r)^2 + (\delta e/e)^2]} \quad (8)$$

where δe/e = δe(11)/e(11) from equation (5) and δr/r is from equation (6).

In dual label counting the efficiency correction has the form

$$R(m) = [e(nn)*r(m) - e(mn)*r(n)] / d \quad (9)$$

where

$$d = e(mm)*e(nn) - e(nm)*e(mn) \quad (10)$$

and e(mn) stands for isotope n efficiency in window m.

Corrected count rate α-sigma percentage error in the dual label case is calculated using the equation

$$\alpha* \delta R(m)/R(m) =$$

$$\alpha*100\sqrt{[[r(m)^2 * \delta e(nm)^2 + e(nm)^2 * \delta r(m)^2 + r(n)^2 * \delta e(mm)^2 + e(mm)^2 *\delta r(n)^2]/p(m)^2+Q/d^2]} \quad (11)$$

where r(m) is from equation (3), δr(m) is from equation (6), d is from equation (10),

$$p(m) = e(nn) * r(m) - e(nm) * r(n) \quad (12)$$

and

$$Q = e(mn)^2 * \delta e(nm)^2 + e(nm)^2 * \delta e(mn)^2 + e(nn)^2 * \delta e(mm)^2 + e(mm)^2 * \delta e(nn)^2 \quad (13)$$

3.3.3.3 Half-life correction

Half-life corrected count rate I is calculated using the equation

$$I_\tau = [[(t/\tau)*\ln(2)]/\{\exp[-\ln(2)*(T-t)/\tau]-\exp[-\ln(2)*(T/\tau)]\}]*R \quad (14)$$

where t is counting time, T is elapsed time from zero time to the end of counting, τ is the half-life of the isotope and R is the background and detector efficiency corrected count rate. Both single and dual label corrections have the same form. Half-life correction has no effect on the corrected count rate errors.

3.3.4 Spectrum indexes

3.3.4.1 Isotope spectrum end point

Isotope spectrum end point (heading ISEP) is a channel at the end of the spectrum.

3.3.4.2 Spectrum quench parameter

Spectrum quench parameter of the isotope spectrum (heading SQP(I)) is the spectrum mean pulse height and is calculated using the equation

$$q = \sum[i * c(i)] / \sum c(i) \quad (15)$$

where i = window 1 low .. Max (window 1 high, window 2 high, window 3 high) is the channel number and c(i) is the number of counts in channel i. The SQP(I) counting window is truncated so that a 10 counts range is removed from both ends.

The α - sigma percentage error of SQP(I) is given by:

$$\alpha*\delta q/q = \alpha*100 \sqrt{(\{\sum[i^2 c(i)]/\sum c(i) - q^2\}/\sum c(i))}/q \quad (16)$$

3.3.4.3 Asymmetric quench parameter

Asymmetric quench parameter (heading AQP(I)) is a measure of the isotope spectrum for an asymmetric sample and varies as the quench changes. It establishes a relation between quench and efficiency.

3.3.5. Standardization

3.3.5.1 Activity standardization

Standardization corrects for differences in sample quench levels and in detector responses.

All standard samples are counted with detector 1. Only the unquenched standard N1 and the fully quenched standard N2 are counted with all detectors. The SQP(I)s or AQP(I)s, efficiencies and weights of standard samples are calculated and stored for activity calculations.

The efficiency in a counting window is

$$E = r/a \quad (17)$$

where r is background and half-life corrected count rate and a is the given activity of the standard sample.

Standardization weight is given by

$$w = \sqrt{[(k*\delta q)^2 + (\delta r/a)^2]} \quad (18)$$

where k is the slope of the standard curve at q, δq is from equation (16) and δr is from equation (6).

3.3.6. Activity

3.3.6.1 Using activity standardization

Sample activity in disintegrations per minute (headings DPM and DPM1) is

$$A = r/E \quad (19)$$

where r is background corrected count rate (3) and E is counting efficiency (17) at SQP(I) or AQP(I). Activity is half-life corrected using equation (14).

Efficiency E is obtained from the standardization efficiency curve of detector 1 using the measured SQP(I) or AQP(I) of detector i.

The measured SQP(I) or AQP(I), q(i), is first corrected to correspond to detector 1,

$$q = q(1,N2) + [\{q(1,N1)-q(1,N2)\}/\{q(i,N1)-q(i,N2)\}]*[q(i)-q(i,N2)] \quad (20)$$

where the first index stands for the detector number and the second index stands for the standard sample type.

If the measured SQP(I) or AQP(I) is not between the standardization SQP(I) or AQP(I) limits, then linear extrapolation with the two first or last points is used (or spline with the two last points if smoothing spline is selected). If the DPM out-of-range monitor (heading DPMM) is selected then OUT is printed to warn the user.

The interpolated efficiency is corrected to correspond to detector i using the equation

$$E = E(i,N2) + [\{E(i,N1)-E(i,N2)\}/\{E(1,N1)-E(1,N2)\}]*[E(1)-E(1,N2] \quad (21)$$

Activity α-sigma percentage error (headings DPM% and DPM1%) is given by

$$\alpha*\delta A/A = \alpha\sqrt{[(\delta r/r)^2 + (\delta E/E)^2]} \quad (22)$$

where $\delta r/r$ is count rate error (6) and efficiency error comes from the equation

$$\delta E = \sqrt{[(k*\delta q)^2 + w^2]}. \quad (23)$$

Here k is the slope of the standard curve and w is the standardization weight at q.

3.3.7 Chemiluminescence correction

Chemiluminescence correction is done by subtracting the chemiluminescence counts from window 1,

$$c(corr) = c - c(chem). \quad (24)$$

Chemiluminescence counts are calculated using the equation:

$$c(chem) = 7.4E\text{-}9 * c(l) * c(r) \quad (25)$$

where c(l) and c(r) are the lower and upper detector counts respectively.

The percentage of chemiluminescence in window 1 (heading CLM%) is given by

$$l = 100 * [c - c(corr)]/c \quad (26)$$

If CPM1 < 10 then chemiluminescence percentage is always zero.

3.3.8 Statistics monitor

If statistics monitoring is selected (heading STM) then the counting time is divided into ten parts and the samples are recounted if bad statistics is detected.

The counting time must be at least ten seconds for the statistics monitor to function. E.g. 53 seconds is divided into 10 times 5 seconds and the last 3 seconds is not monitored. ., REC1, REC2 or ERR is printed for samples with no recounting, one recounting, two recountings or two recountings and still bad statistics. Using the next sample function disables the statistics monitor. If counting ends because of precision then the statistics monitor functions only if at least nine parts have already been counted.

Statistics is defined as bad if the chi-square value

$$\chi^2 = O^2/T^2 = [\{\sum i^2 - n * i(mean)^2\}/\{n-1\}] /[(1/n)\sum(i/t)]>2.4 \quad (27)$$

Here O is the observed deviation and T is the theoretical deviation. The count rate during time part t is i and the number of parts n = 10.

3.3.9 Statistical calculations

3.3.9.1 Mean

The mean value of corrected count rate or activity A from n repeats or replicates (headings MEAN, MEAN1, MEAN2) is calculated using the equation

$$M = \sum A / n \quad (28)$$

where the sum is over the repeat or replicate number.

3.3.9.2 Theoretical standard error of mean

The theoretical percentage standard error T of the mean M (headings THEOR.ERROR(%), TSEM1%, TSEM2%) is calculated using the equation

$$T = \alpha*100 \sqrt{[\sum(\delta A)^2]} / (n * M) \quad (29)$$

where δA is the corrected count rate or activity α-sigma error.

3.3.9.3 Standard error of mean

The observed standard error O of the mean M in percentages (headings OBS.ERROR(%), SEM1%, SEM2%) is calculated using the equation:

$$O = \alpha*100\sqrt{\{\sum(M-A)^2/[n(n-1)]\}}/M \quad (30)$$

$$= \alpha*100\sqrt{\{[\sum A^2 - (\sum A)^2/n]/[n(n-1)]\}}/M$$

3.3.9.4 Theoretical coefficient of variation

The theoretical coefficient of variation CV% of the mean M in percentages (headings TCV1%, TCV2%) is calculated using the equation:

$$TCV\% = \alpha*100\ \sqrt{\{[\ \sum\ (\delta A)^2]/n\}}/M \quad (31)$$

3.3.9.5 Coefficient of variation

The observed coefficient of variation CV% of the mean M in percentage (headings CV1%, CV2%) is calculated using the equation:

$$CV\% = \alpha\ *100\sqrt{\{\sum(M-A)^2/(n-1)\}}/M \quad (32)$$

$$= \alpha\ *100\sqrt{\{[\sum A^2 - (\sum A)^2/n]/(n-1)\}}/M$$

3.3.9.6 Theoretical standard deviation

The theoretical standard deviation TSD (headings TSD1 and TSD2) is calculated using the equation:

$$TSD = \alpha*\sqrt{\ [\sum(\delta A)^2/n]} \quad (33)$$

3.3.9.7 Observed standard deviation

The observed standard deviation SD (headings SD1 and SD2) is calculated using the equation:

$$SD = \alpha*\sqrt{\{[\sum A^2 - (\sum A)^2/n]/(n-1)\}} \quad (34)$$

3.3.9.6 Chi-square test

The reduced chi-square value χ^2 (headings CHI-SQUARED, CHISQ1, CHISQ2) is defined as

$$\chi^2 = O^2 / T^2 \quad (35)$$

where the observed error O is from equation (30) and the theoretical error T is from equation (29).

How much χ^2 deviates from unity is a direct measure of the extent to which O deviates from T. With a limited number of measurements we would not expect these two errors to be precisely the same. Thus a more quantitative test is required to determine whether the observed difference between these two deviations is really significant. For this purpose we can apply the chi-square test.

From the χ^2-value we can calculate the probability (headings PROBABILITY(%), P1, P2) that a random sample from a correct distribution would have a larger value of χ^2 than the observed χ^2-value.

3.4 Specifications

3.4.1 Physical dimensions

	TriLux	JET
Width	430 mm	700 mm
Height	610 mm	630 mm
Depth	645 mm	660 mm
	655 mm 12 det.	
Weight	85 kg	90 kg
	90 kg 12 det.	

3.4.2 Power

Mains voltage selectable 100, 115, 120, 220, 240 V, +/- 10 %, 50/60 Hz, power consumption 360 VA.

Injector module: 24 V +/- 10 %, max 2A.

3.4.3 External power supply

Power failure recovery and protection can be obtained with an external UPS back-up power supply provided. This can be ordered from PerkinElmer Life and Analytical Sciences.

3.4.4 Connections/input/output

Serial ASCII interface RS-232 C. Connector No.1 for terminal computer, No.2 for mainframe or data net and No.3 for printer.

3.4.5 Operating conditions

Temperature +15 to +35^{o}C.
Humidity max. 10 - 80 %.
Pollution degree 2.
Installation category II.
Altitude 2000 m.

3.4.6 Sample capacity

Sample capacity using 96-well sample plates is 16(32)*96 = 1536(3072) samples, using 24-well sample plates 16(32)*24 = 384(768) samples and using 384-well sample plates 16(32)*384 = 6144(12288). Any combination of cassettes either for 96 or 24-well sample plates or 96 or 384-well sample plates is possible.

3.4.7 Sample cassette changer

A z-direction moving cassette rack with shelves for 16 or 32 cassettes. This rack functions as a random access stack which allows access to any cassette in the rack. The cassette rack is driven by a stepper motor and its movement is controlled by electro-optical sensors. A transportation system moving cassettes in the x-y-direction selects one cassette from the cassette rack and takes it into the counting position and then returns the cassette to the cassette rack. This transportation system is driven by two stepper motors and is controlled by electro-optical sensors.

3.4.8 Mask adapter (Injector assembly)

A transportation system for moving the mask adpater in the x-direction from the maintenance tray into the counting chamber under the upper detector block. This transportation system is driven by one stepper motor and is controlled by electro-optical sensors. The mask adapter moves in the z-direction with the upper detector block.

3.4.9 Detector assembly

1, 2, 3, 6 or 12 detectors (each consisting of a pair of 19 mm PM tubes working in coincidence), are mounted in two detector assemblies, the upper detector assembly moving in the z-direction. PM tubes are Hamamatsu R1166 or equivalent. Coincidence resolution time is 100 ns. One to six detector assemblies are provided with circular apertures for 96 and 24-well sample plates. The 12-detector assembly is provided with circular apertures for 96-well sample plates and square apertures for 384-well sample plates. The apertures are automatically changed to correspond to the plate type.

3.4.10 Cassettes

Cassettes are made of special-grade polycarbonate plastic containing a high amount of white pigment to produce a very high degree of reflectivity (about 90 %) while eliminating optical crosstalk between wells. Measurement is made through the cassettes (pat. pend.).

3.4.10.1 96-pos. cassette 1450-101

This has 96 sample holes with a diameter of 7.8 mm. Well separation is 9 mm. The cassette is used when counting 1450-401 (or equivalent) 96-well sample plates.

3.4.10.2 24-pos. cassette 1450-102

This has 24 sample holes with a diameter of 13.2 mm. Well separation is 18 mm. This cassette is used when counting 1450-402 (or equivalent) 24-well sample plates.

3.4.10.3 96-pos. cassette 1450-103

This has 96 sample holes. The cassette is used when counting 1450-407 plates.

3.4.10.4 96-pos. cassette 1450-104

This is a two piece cassette with 96 sample holes. This cassette is used when counting filtermats. The filtermat in a sample bag is placed between the base plate and the cover plate.

3.4.10.5 96-pos. cassette 1450-105

This has 96 sample holes. This cassette is used when counting 1450-410, 1450-405, 1450-419 or equivalent sample plates.

3.4.10.6 96-pos. cassette 1450-106

This has 96 sample holes. This cassette is used when counting e.g. Millipore MultiScreen™ Filtration plates or equivalent sample plates.

3.4.10.7 24-pos. cassette 1450-107

This is a two piece cassette with 24 sample holes. The cassette is used when counting Skatron filtermats cut into four pieces. The filtermat in a sample bag is placed between the two plates of the cassette.

3.4.10.8 24-pos. cassette 1450-110

This is an open-based cassette. The cassette is used when counting 24 well culture plates, e.g. Costar plates (or equivalent).

3.4.10.9 24-pos. cassette 1450-116

This is a two piece cassette with 24 sample holes. This cassette is used when counting filtermats. The filtermat in a sample bag is placed between the base plate and the cover plate.

3.4.10.10 24-pos. cassette 1450-117

This is a two piece cassette with 24 sample holes. This cassette is used when counting 4 mL sample vials. The vials are placed between the base plate and the cover plate.

3.4.10.11 96-pos. cassette 1450-118

This is a two piece cassette with 96 sample holes. This cassette is used when counting e.g. ^{32}P-labelled dot blot samples from filtermats such as 1450-423 Nylon membrane. No scintillator needs to be added to the filtermat allowing reprobing assays. The filtermat in a sample bag is placed between the base plate and the cover plate having solid scintillator in sample positions.

3.4.10.12 384-pos. cassette 1450-130

This has 384 sample holes. the cassette is used when counting 384-well sample plates.

All the cassettes are made of white plastic with an optical surface in the sample holes.

3.4.11 Sample identification system

Each cassette can be provided with a 1450-451 ID Support Plate for 1450-452 ID Labels. An ID Support Plate has marked areas for ASSAY, PROTOCOL and CASSETTE numbers (0 - 99) and FUNCTION codes (CPM-normalization, DPM-standardization, stop). This provides the information needed for Good Laboratory Practice.

There is also an optional Plate ID reader.

3.4.12 Electronics

3.4.12.1 Environmental

Electromagnetic susceptibility according to: EN50082-1.

Electromagnetic emissions according to: EN500081-1.

The following interconnecting cables should be used to comply with these standards:
Instrument to PC: 1221-503
Instrument to printer: 1221-502

3.4.12.2 Hardware

16-bit microprocessor controlled counting and data reduction. Memory configuration 64 kbyte ROM and 1 Mbyte RAM. Two 3.5 inch 1.44 Mbyte disk drives: drive A for program disk and drive B for protocol disk.

Logarithmic A/D converter energy range 1 - 2000 keV. There is a dual 1024-channel multichannel analyzer for each detector. Built-in dead time correction.

3.4.13 Performance

(100 ns coincidence time)

3.4.13.1 Background

(at the factory, Turku, Finland):
With empty 1450-514 plate:
<15 CPM in wide ^{3}H window
<17 CPM in wide ^{14}C window.

Unquenched sample with a volume of 150 microlitres high flash point cocktail unpurged in the 1450-401 96-well Sample Plate: typically 6 CPM in wide ^{3}H window typically 9 CPM in wide ^{14}C window.

Unquenched sample with a volume of 750 microlitres high flash point cocktail unpurged in the 1450-402 24-well Sample Plate:
typically 24 CPM in wide ^{3}H window
typically 37 CPM in wide ^{14}C window.

3.4.13.2 Efficiency

1450-471 Normalization Standard
^{3}H > 45 %
^{14}C > 90 %

Unquenched sample with a volume of 150 microlitres high flash point cocktail unpurged in the 1450-401 96-well Sample Plate:
^{3}H: typically 57 %
^{14}C: typically 94 %

Unquenched sample with a volume of 750 microlitres high flash point cocktail unpurged in the 1450-402 24-well Sample Plate:
^{3}H: typically 61 %
^{14}C: typically 95 %

3.4.13.3 Crosstalk

Optical crosstalk due to light piping in the plastic of the sample plates. The values are given for sample plates with black printing inhibiting crosstalk between the sample wells.

Unquenched sample with a volume of 150 microlitres high flash point cocktail unpurged in the 1450-401 96-well Sample Plate:
In wide ^{3}H window: < 0.05 %
In wide ^{14}C window: typically 1.8 %

Unquenched sample with a volume of 750 microlitres in the 1450-402 24-well Sample Plate:

In wide ^{3}H window < 0.02 %
In wide ^{14}C window: typically 0.2 %

3.4.13.4 Stability

Count variation less than 0.5 % / 24 hours (not including random statistics).

3.4.14 Program Specifications

3.4.14.1 Counting protocols

100 fully programmable counting protocols. Start of counting manually through keyboard command or automatically by the use of ID labels.

Delay Start, allows for sample incubation prior to start of measurement.

Edit, Copy, Print, Delete, List features for management of protocols.

Password protection against unwanted changes of protocol.

Help function, built-in operating instructions supports the user throughout the program.

Single label CPM or DPM counting.

Dual label CPM or DPM counting.

Crosstalk correction to allow different types of microtitration plate to be used.
Scintillation proximity assay can be done.

Preset parameter for 6 common isotopes.

Count termination by counting time or reached precision (2 sigma error).

Plate counting control, whole plate or only active rows or positions.

Default, Short or Long printer output for quick printout settings.

Fully programmable printout selection. Including statistical analysis of results and user functions.

Fully programmable output to display. Including statistical analysis of results and user functions.

Fully programmable output to mainframe. Including statistical analysis of results and user functions.

Fully programmable output to file on terminal PC. Including statistical analysis of results and user functions.

Repeat or Replicates counting.

Cycle counting, repeat counting of the whole assay.

Background correction. CPM/DPM results can be corrected for experimental background either by typed in background value or background samples.

Programmable counting time for background samples.

Half-life correction, corrects the CPM/DPM values for decay of short-lived isotopes. Zero time start of assay or specified date and time.

Chemiluminescence correction, corrects the CPM/DPM values for random coincidence contributions.

Statistics Monitor, detects distorting contributions from static electricity.

Static electricity discharge is a phenomenon which occurs together with the use of plastic sample carriers such as microtitration plates.

3.4.14.2 Normalization protocols

Up to 100 Normalization protocols/data sets. Normalization of the detectors is required to eliminate possible difference in counting efficiency between the detectors. Normalization is done for the different isotopes, different applications or different window settings. The Normalization protocol to be used is selected in the counting protocol.

Also available in the protocol are:

Half-life correction.
Chemiluminescence correction.
Password protection.

3.4.14.3 Standardization protocols

Up to 100 Quench standardization protocols/data sets. Quench standardization is needed to correct for counting efficiency variation or quench in the samples. The Quench Standardization protocol to be used is selected in the counting protocol.

Selection of curve fit method; smoothed spline, interpolation spline, linear interpolation or linear regression.

Input of individually adjusted DPM values for the standard samples.

Also available in the protocol are:

Chemiluminescence correction.
Half-life correction.
Password protection.

3.4.14.4 MicroBeta JET injector system

One injector module dispensing at a time. Reading before dispensing, reading after dispensing and delay time after dispensing.

3.4.15 Consumables

3.4.15.1 Sample Plates

1450-401 96-well Sample Plate (25/box): 96 round-bottomed wells, 8x12 format, made of clear PET-plastic, printed lines between wells, chemically resistant to all HiSafe-cocktails, max. volume 250 µL/well. Sealed with tape type 1450-461 or 1450-462 or heatsealed with 1450-463 (in 1495-021 Microsealer). Counted in cassette type 1450-101.

1450-402 24-well Sample Plate (25/box): 24 flat-bottomed wells, 4x6 format, made of clear PET-plastic, printed lines between wells, chemically resistant to all HiSafe-cocktails, max. volume 1 mL/well. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-102.

1450-405 96-well Sterile Sample Plate (10/box): 96 flat-bottomed wells, 8x12 format, made of polystyrene, white well walls and clear well bottom, sterile tissue culture-grade, with a lid, max. volume 350 µL/well. For tissue culture samples and for samples with Polystyrene Safe cocktail 1450-444. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-105.

1450-407 96-well Sample Plate, PS, 400 µL (25/box): 96 flat-bottomed wells, 8x12 format, made of clear polystyrene, max. volume 400 µL /well. For aqueous samples (SPA) or for samples with Polystyrene Safe cocktail 1450-444. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-103.

1450-408 24-well Heat-sealable Sample Plate (25/box): 24 flat-bottomed wells, 4x6 format, made of clear PET-plastic, printed lines between wells, chemically resistant to all HiSafe-cocktails, max. volume 1 ml/well. Sealed with tape type 1450-461 or 1450-462 or heatsealed with 1450-463 (in 1495-021 Microsealer using 1495-023 Heat Seal Support 24). Counted in a cassette 1450-102.

1450-410 96-well Rigid Sample Plate (25/box): 96 flat-bottomed wells, 8x12 format, made of polystyrene, white well walls and clear well bottom, max. volume 350 µL/well. For aqueous samples (e.g. SPA) or for samples with Polystyrene Safe cocktail 1450-444. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-105.

1450-419 ScintiStrip™ (120/box): 12 breakable strips of 8 wells on a support frame 1450-482, made of scintillation plastics, max. volume 300 µL/well. For immobilised samples or aqueous samples, no scintillation cocktail needed! Sealed with tape type 1450-461 or 1450-462. Counted on the support frame in cassette type 1450-105.

1450-514 96-well Isoplate™ (25/box): 96 flat-bottomed wells, 8x12 format, made of clear polystyrene wells with a rigid white frame, max. volume 350 µL /well. For aqueous samples (e.g. SPA) or for samples with SuperMix cocktail 1200-439. Sealed with tape type 1450-461 or 1450-462. Counted in cassette type 1450-105.

3.4.15.2 Filtermats

1450-421 Printed Filtermat A (100/box): 96-position glassfibre filter mat with printed pattern. For harvested cell and receptor samples. Scintillant to be used: Betaplate Scint 1205-440 or MeltiLex B/HS 1450-442. Placed in a sample bag type 1450-432. Counted in cassette type 1450-104.

1450-422 Filtermat A (100/box): 24-position glass -fibre filter mat with printed pattern. For harvested cell and receptor samples. Scintillant to be used: Betaplate Scint 1205-440 or MeltiLex A 1450-441. Place in a sample bag 1450-432. Counted in cassette type 1450-116.

1450-423 Printed Nylon Membrane (50/box): 96-position nylon membrane with printed pattern; for DNA samples. Scintillant to be used: Betaplate Scint 1205-440 or MeltiLex A 1450-441. Placed in sample bag type 1450-432. Counted in cassette type 1450-104.

1450-424 Filtermat B (50/box): 24-position filtermat with printed pattern made of glassfibre. For harvested or pipetted samples. Scintillant to be used: Betaplate Scint 1205-440 or MeltiLex B/HS 1450-442. Place in a sample bag 1450-432. Counted in cassette type 1450-116.

1450-521 Printed Filtermat B (50/box): 96-position glassfibre filter mat with printed pattern. For harvested receptor or cell samples. Scintillant to be used: Betaplate Scint 1205-440 or MeltiLex A 1450-441. Placed in sample bag type 1450-432. Counted in cassette type 1450-104.

1450-522 DEAE Filtermat (100/box): 96-position filter mat with printed pattern made of glassfibre, containing positively charged groups. For harvested or pipetted (negatively charged) samples. Scintillant to be used: Betaplate Scint 1205-440 or MeltiLex A 1450-441. Placed in sample bag type 1450-432. Counted in cassette type 1450-104.

1450-523 P30 Filtermat (100/box): 96-position filter mat with printed pattern made of glassfibre, containing negatively charged groups. For harvested or pipetted (positively charged) samples. Scintillant to be used: Betaplate Scint 1205-440 or MeltiLex A 1450-441. Placed in a sample bag 1450-432. Counted in a cassette type 1450-104.

3.4.15.3 Plastic Bags

1450-431 Waste Bag (100/box): Waste bag for used sample plates. Chemically resistant to safe cocktails. Closed with a heatsealer (1295-012).

1450-432 Sample Bag (100/box): Sample bag for filters 1450-421 and 1450-423. Chemically resistant to safe cocktails. Closed with a heatsealer (1295-012).

3.4.15.4 Scintillation Products

1450-419 ScintiStrip™ (120/box): 12 breakable strips of 8 wells on support frame type 1450-482, made of scintillation plastics, max. volume 300 µL/well. For immobilised samples or aqueous samples, no scintillation cocktail needed! Sealed with tape type 1450-461 or 1450-462. Counted on the support frame in cassette type 1450-105.

1450-441 MeltiLex A (100/box): Meltable solid scintillator for filters 1450-421 and 1450-423 or other filters. Melting temperature +60..+120°C.

1450-442 MeltiLex B/HS (50/box): Meltable solid scintillator for filters 1450-521, 1450-424 and other thick filters. Melting temperature +60..+120°C.

1450-444 Polystyrene Safe (5L/box): Scintillation cocktail to be used with polystyrene sample plates, e.g. 1450-405, 1450-407 and 1450-410.

3.4.15.5 ID Products

1450-451 ID Support Plate: Support plate for ID labels. Placed onto a counting cassette.

1450-452 ID Labels (10 sheets/box): Labels for identifying Assay number (necessary for MultiCalc assays), Protocol and Cassette number and Function code. Fixed on a Support Plate 1450-451.

3.4.15.6 Tapes

1450-461 Sealing Tape (100/box): Sealing tape for sample plates, permanent.

1450-462 Removable Sealing Tape (100/box): Sealing tape for sample plates; removable.

1450-463 Heatsealing Foil (100/box): Sealing foil for sample plates type 1450-401 and 1450-408. Heatsealed with a 1495-021 Microsealer.

1450-465 Printed Sealing Tape (100/box): Sealing tape for sample plates, printed.

1450-466 Coloured Sealing Tape (100/box): Sealing tape for luminescent sample plates.

3.4.15.7 Other Consumables

1450-481 Support Frame (25/box): Support frame for sample plates type 1450-401, 1450-402 and 1450-408. Removed before counting.

1450-482 Support Frame for 1450-407 (25/box): Support frame for sample plates type 1450-407 and 1450-419. Removed before counting 1450-407. 1450-419 ScintiStrips are counted with the support frame.

1450-486 MicroBeta Starter kit, MicroPlates: Contains the MicroBeta sample plates (5 of each), tapes and heat sealable foil.

1450-487 MicroBeta Starter kit, Filters, Membranes and Solid Scintillators: Contains the MicroBeta filters, membranes, MeltiLex-sheets, Sample bags and Waste bags.

3.4.15.8 Adapters and Inserts

1450-108 Adapter: For Eppendorf tubes (or equivalent). The adapters are placed on a 1450-102 cassette, a bottom tape is used if needed.

1450-109 Insert: For 24-well cell culture plates. The inserts are placed in the sample wells in order to reduce crosstalk between the wells.

3.5 Abbreviations and acronyms used

.	Sample passed monitor tests (monitor output)
A/D	Analogue to digital
ABS	Absolute value [1]
ALT	Alternate key
AQP(I)	Asymmetric quench parameter of the isotope spectrum
AQP(I)%	Theoretical percentage error of AQP(I)
AS	Value of assay ID for the current plate [1]
ASCII	American Standard Code for Information Interchange
BDH	Laboratory reagent and equipment sales company
BG	Background
BGND	Background
BGNDm	Background in window m [2]
BIOS	Basic Input Output System
Bk	Background
bps	Bits per second
BS	Backspace key
CA	Value of cassette ID for the current plate [1]
CASS	Cassette field in ID support plate
CCPM	Corrected count rate (counts per minute)
CCPM%	Theoretical percentage error for CCPM
CCPM1	Corrected count rate (counts per minute) for isotope 1 [1]
CCPM1%	Theoretical percentage error for CCPM1 [1]
CCPM2	Corrected count rate (counts per minute) for isotope 2 [1]
CCPM2%	Theoretical percentage error for CCPM2 [1]
CCPM3	Corrected count rate (counts per minute) for window 3 [1]
CCPM3%	Theoretical percentage error for CCPM3 [1]
CCPS1	Corrected count rate (counts per second) for isotope 1 [1]
CCPS2	Corrected count rate (counts per second) for isotope 2 [1]
CCPS3	Corrected count rate (counts per second) for window 3 [1]
CGA	Colour Graphics Adapter
ch	Channel
CHISQ1	Reduced chi-square value for CCPM1, DPM1 or LCPS [1]
CHISQ2	Reduced chi-square value for CCPM2 or DPM2 [1]
CLM%	Chemiluminescence in percentages in window 1 [1]
CNO	Cassette order number [1]
COUNTS1	Counts for isotope 1 [1]
COUNTS2	Counts for isotope 2 [1]
COUNTS3	Counts for window 3 [1]

cpi	Characters per inch
CPM	Count rate (counts per minute)
CPM1	Count rate (counts per minute) for isotope 1 [1]
CPM1%	Theoretical percentage error for CPM1 [1]
CPM2	Count rate (counts per minute) for isotope 2 [1]
CPM2%	Theoretical percentage error for CPM2 [1]
CPM3	Count rate (counts per minute) for window 3 [1]
CPM3%	Theoretical percentage error for CPM3 [1]
CPM_C	Coincidence count rate (counts per minute)
CPM_NC	Non-coincidence count rate (counts per minute)
CPM_AQP	AQP(I) count rate (counts per minute)
CPS1	Count rate (counts per second) for isotope 1 [1]
CPS2	Count rate (counts per second) for isotope 2 [1]
CPS3	Count rate (counts per second) for window 3 [1]
CT	Cassette type [1]
CTIME	Dead time corrected counting time in seconds [1]
CTRL	Control key
CV1%	Percentage coefficient of variation for CCPM1, DPM1 or LCPS 1
CV2%	Percentage coefficient of variation for CCPM2 or DPM2 [1]
D	Detector number [1]
DATE	Current date [1]
DCCPM1	Absolute theoretical error for CCPM1 [1]
DCCPM2	Absolute theoretical error for CCPM2 [1]
DCCPM3	Absolute theoretical error for CCPM3 [1]
DDPM1	Absolute theoretical error for DPM1 [1]
DDPM2	Absolute theoretical error for DPM2 [1]
DEAE	Diethylaminoethyl
DEL	Delete key
DET	Detector number [2]
DIN	Di-isopropylnaphthalene
DLCPS	Absolute theoretical error for LCPS [1]
DNA	Deoxyribonucleic acid
DPM	Activity (disintegration per minute)
DPM%	Theoretical percentage error for DPM
DPM1	Activity (disintegration per minute) for isotope 1 [1]
DPM1%	Theoretical percentage error for DPM1 [1]
DPM2	Activity (disintegration per minute) for isotope 2 [1]
DPM2%	Theoretical percentage error for DPM2 [1]
DPMM	DPM out of range monitor [1]
DTR	Flow control using Data Terminal Ready line

EEC	European Economic Community
EFF	Counting efficiency [2]
EFF1	Counting efficiency for isotope 1 [1]
EFF2	Counting efficiency for isotope 2 [1]
EFFmn	Counting efficiency, window m, isotope n [2]
EGA	Enhanced Graphics Adapter
ERR	Variation after two recounts (statistics monitor output)
ETIME1	Elapsed time in hours for isotope 1 from zero time 1 [1]
ETIME2	Elapsed time in hours for isotope 2 from zero time 2 [1]
EXCL	Controls excluding of unrequired parts of the output [1]
EXP	Exponent function [1]
FF	Formfeed character to the printer after 'End of assay'-message [1]
FLAG	Termination flag [1]
FU	Value of function ID code for the current plate [1]
FUNC	Function field in ID support plate
h	Hour
HIGH	Too high count rate (luminescence monitor output)
IAEA	International Atomic Energy Agency
IBM	International Business Machines
ID	Identifier
INT	Counting interrupted (termination flag)
ISEP	Isotope spectrum end point [1]
ISEP%	Theoretical percentage error for ISEP [1]
ISO	International Standards Organization
ISOT	Isotope
kB	kilobyte
kBq	kiloBequerel
keV	kiloelectronVolt
LAN	Local area network
LCPS	Luminescence corrected count rate (counts per second) divided by 100 [1]
LCPS%	Theoretical percentage error for LCPS [1]
LM	LCPS out of range monitor [1]
LN	Natural logarithm [1]
LSC	Liquid scintillation counter
m	Minute
MAX	Maximum counts per channel reached (termination flag)
MB	MicroBeta (starts program from DOS); Megabyte
MC	MultiCalc
MCA	Multichannel analyzer
MCGA	Multicolour Graphics Array

MEAN1	Mean value for CCPM1, DPM1 or LCPS [1]
MEAN2	Mean value for CCPM2 or DPM2 [1]
MS-DOS	Microsoft Disk Operating System
N	No
nCi	nanoCurie
NEWLINE	Start a new line [1]
NL	Start a new line [1]
NO	Number
NOLIVE	No status (live) display [1]
NORM	Normalization ID label
OUT	Out of range (DPM monitor output)
P30	Ortho phosphate
P1	Probability in percentages for CCPM1, DPM1 or LCPS [1]
P2	Probability in percentages for CCPM2 or DPM2 [1]
PBS	Phosphate buffered saline
PC	Personal computer
PET	Polyethylene terephtalate
PLATE	Output results in microtitration plate format [1]
PLATEID	Plate identifier
PMT	Photomultiplier tube
POS	Sample position on microtitration plate [1]
PR	Protocol number used [1]
PREC	Precision reached (termination flag)
PRINTER	Output same data as specified for printer [1]
PROT	Protocol listing [1]; Protocol field in ID support plate
PS/2	Personal System/2
PVC	Polyvinyl chloride
RAM	Random access memory
REC1	Recounted once (statistics monitor output)
REC2	Recounted twice (statistics monitor output)
REPL	Replicate sample range [1]
RESP1	Response (CCPM1 or LCPS) [1]
RESP1%	Theoretical percentage error for RESP1 [1]
RESP2	Response (CCPM2) [1]
RESP2%	Theoretical percentage error for RESP2 [1]
ROM	Read only memory
RP	Repeat number of the current measurement [1]
RS-232C	Recommended standard number 232, revision C, for data communications
s	Second
S	Spectrum half [1]

S1	Selects 1 sigma level for all deviation output fields [1]
SD1	Standard deviation for CCPM1, DPM1 or LCPS [1]
SD2	Standard deviation for CCPM2 or DPM2 [1]
SEM1	Standard error of mean for CCPM1, DPM1 or LCPS [1]
SEM1%	Percentage standard error of mean for CCPM1, DPM1 or LCPS [1]
SEM2	Standard error of mean for CCPM2 or DPM2 [1]
SEM2%	Percentage standard error of mean for CCPM2 or DPM2 [1]
SEQ	Running sequential sample number [1]
SH	Shelf number [1]
SPA	Scintillation proximity assay
SPECTRA	Plot spectra [1]
SPSAVE	Save spectra to file in compressed numerical format [1]
SQP(I)	Spectrum quench parameter of isotope spectrum [1]
SQP(I)%	Theoretical percentage error for SQP(I) [1]
SQP(I)m	SQP(I) for isotope m [2]
SQRT	Square root [1]
STAT	Data specified will be output when a statistics mean is available [1]
STD	Standardization ID label
STIME	Counting start time [1]
STM	Statistics monitor [1]
STOP	Stop ID label
TAB	Produces tab-character between all output columns [1]
TCA	Trichloroacetic acid
TCV1%	Percentage theoretical coefficient of variation for CCPM1, DPM1 or LCPS [1]
TCV2%	Percentage theoretical coefficient of variation for CCPM2 or DPM2 [1]
TIME	Current time [1]
TSD1	Theoretical standard deviation for CCPM1, DPM1 or LCPS [1]
TSD2	Theoretical standard deviation for CCPM2 or DPM2 [1]
TSEM1	Theoretical standard error of mean for CCPM1, DPM1 or LCPS [1]
TSEM1%	Percentage theoretical standard error of mean for CCPM1, DPM1 or LCPS [1]
TSEM2	Theoretical standard error of mean for CCPM2 or DPM2 [1]
TSEM2%	Percentage theoretical standard error of mean for CCPM2 or DPM2 [1]
TXT	Produces 'Start of assay:nn'-message [1]
UN	United Nations
UTMAC	UltroTerm for Macintosh
VGA	Video Graphics Array
VT100	Digital Equipment Corporation video terminal type
VT52	Digital Equipment Corporation video terminal type
W1	Window 1 limits [1]
W2	Window 2 limits [1]
W3	Window 3 limits [1]

WT Wallac terminal emulation
XON/XOFF Flow control using XON (ASCII 17) and XOFF (ASCII 19) characters
Y Yes

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[1] Programmable output
[2] Normalization and standardization tables

PerkinElmer life sciences.

DECLARATION OF CONFORMITY FOR CE-MARKING

We

Supplier's name
WALLAC OY
Address
PL 10, 20101 TURKU, FINLAND

declare under our sole responsibility that the product

Name, type or model, lot, batch or serial number, possibly sources and numbers of items	
1450 MicroBeta TriLux	Valid from serial number 4500762
1450 MicroBeta JET	Valid from serial number 4500877

to which this declaration relates is in conformity with the following standard(s) or other normative document(s)

Title and/or number and date of issue of the standard(s) or other normative document(s)
EN 50082-1 :1992; EN 50081-1 :1992 EN 61000-3-2 : 1995 + A1:1998 + A2:1998 + A14:2000; EN 61000-3-3:1995 EN 61010-1 :1993

(if applicable) following the provisions of the following directives

Electromagnetic compatibility (EMC), 89/336/EEC Low voltage (LV), 73/23/EEC

Date and place of issue
27 December 2000 TURKU, FINLAND
Name and signature or equivalent marking of authorized person
Pekka Mäkinen, Quality Assurance Manager

Part 4 Installation information

4.1 Installation instructions

Note: Except for where noted these instructions are valid for both MicroBeta TriLux and MicroBeta JET.

4.1.1 Environment

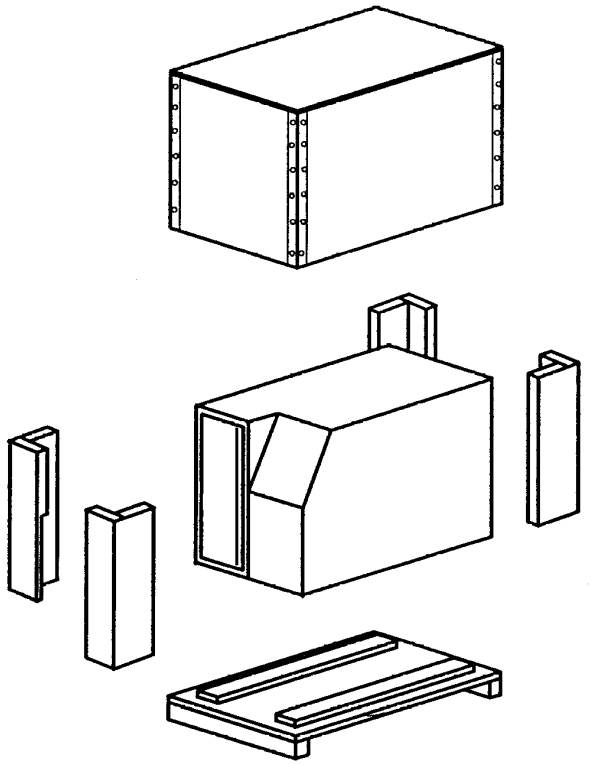
Although normal clean laboratory conditions are usually quite satisfactory as an operational environment it is useful to take the following points into consideration.

If possible a separate room should be provided for the MicroBeta LSC as this allows the best control over the immediate environment. Ventilation in the room should be adequate for all conditions of use, the temperature should be reasonably constant at about 22^{o}C, relative humidity should not be excessive, and direct sunlight should not be able to reach the instrument. It is also important that the various isotopes are stored well away from the instrument in another room. Only those radioactive samples that are actually measured should be in the laboratory at any time in order to keep the background at a low level.

4.1.2 Electric power

Three electrical outlets each having a protective earth should be available, with, if possible, a separate power line for the instrument itself having an isolation switch and a fuse box. If excessive fluctuations in the mains voltage are anticipated, a mains stabilizer may be necessary.

4.1.3 Unpacking



Removing MicroBeta from its packaging

Cut off and remove the binding bands

Open the clinching nails and lift the cover off from the base of the package, see the figure in the previous column.

Unpack all units and accessories and check them according to the packing list also noting any possible transport damage.

Open the lower clinching nails and remove the packing around the main instrument.

Lift the instrument up from the bottom of the package.

Move the instrument to its place of operation.

4.1.4 Checking the mains voltage setting

Measure and note the mains voltage at the outlets to be used.

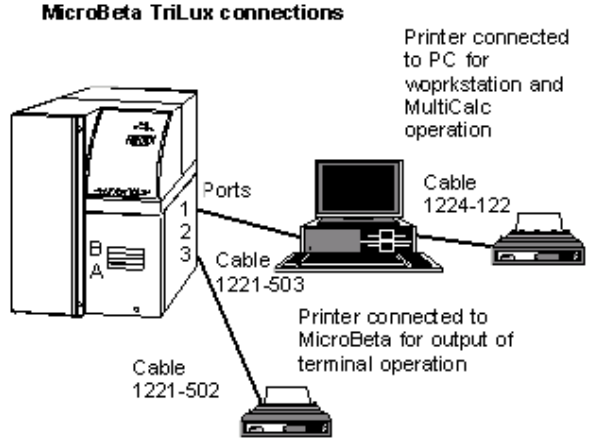
Locate the mains selector switch, this is on the right side when looking from the rear of the instrument. If necessary adjust the mains selector switch to correspond with the measured supply. For supplies with a nominal voltage of 230 V it is recommended that the selector be set to 240 V.

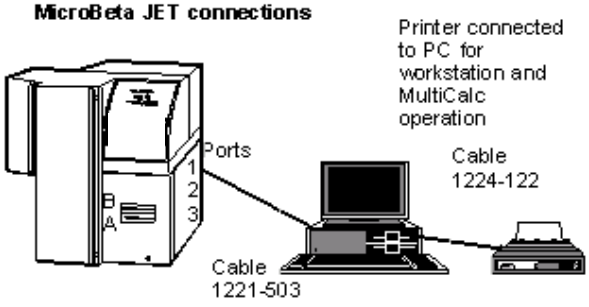
Check that the fuses fitted in the fuse carriers on the back panel are of the correct rating for the local supply, and according to the label - T 4 A for supplies with a voltage in the range 100 to 120 V and T 2 A for supplies with a voltage in the range 220 to 240 V. ***For continued protection against fire hazard, replace only with the same type and rating of fuse.***

Insert the battery fuse in its holder into the socket at the rear of the instrument.

4.1.5 Connecting up the counter and peripherals

Connect the counter to the PC and the printer using the cables shown in the figure.





There are two configurations, either the printer and PC are each connected to MicroBeta to ports 3 and 1 respectively (not possible with MicroBeta JET) or the printer is connected to the PC which is in turn connected to port 1. This latter configuration is for use with the Windows workstation and MultiCalc.

See the additional section at the end of the chapter for information about Installation of the Injector system to MicroBeta JET.

Plug in the power cables for each device.

4.1.6 Start-up

Although the UltroTerm terminal emulator program is the default emulator for MicroBeta, other emulators, e.g. GenTerm and MultiCalc, or VT52 and VT100 terminals can also be used. The following two sections describe how to start MicroBeta with the default UltroTerm and with another emulator.

4.1.6.1 Start-up using UltroTerm

This is the default setting and therefore no parameters must usually be changed in the counter.

1. Switch on the printer.

2. Switch on the PC.

3. Install UltroTerm as described in the UltroTerm User Manual and in section 2.31.5.3 Installation in this manual.

4. Start UltroTerm as described in the UltroTerm User Manual and in section 2.31.5.4 Starting UltroTerm in this manual.

5. If necessary, change the communication parameters as described in the UltroTerm User Manual and in section 2.31.5.5 in this manual.

6. Insert the MicroBeta program disk into drive A:, the lower disk drive of the counter.

7. Insert the MicroBeta protocol disk into drive B:, the upper disk drive of the counter.

8. Switch on the counter with the power switch on the back of it.

9. After about 3 minutes Ready is displayed and the counter is ready for operation.

4.1.6.2 Start-up using another terminal emulator

When the program is started it will check what kind of terminal or emulator is connected to the counter and change its own settings according to this. It will also test the baud rate and the number of data bits and make necessary changes. The program can identify the same emulators as are available in the counter, i.e. GenTerm WT, UltroTerm, MultiCalc VT52, standard VT52 and VT100. It can also identify baud rates from 300 to 9600 bps and 7 or 8 data bits.

Together with changing emulator and communication parameters, the program will also notify the user that the change has been made and that the user must check and correct the terminal and/or RS settings in System parameters. The program will usually work fine with the automatic settings, but it is recommended that the user checks and corrects the system parameters.

1. Switch on the printer.

2. Switch on the PC.

3. Start your terminal emulator.

4. If necessary, change the communication parameters in the terminal emulator.

5. Insert the MicroBeta program disk into drive A:, the lower disk drive of the counter.

6. Insert the MicroBeta protocol disk into drive B:, the upper disk drive of the counter.

7. Switch on the counter with the power switch on the back of the counter.

8. After about 2 minutes a message like the following will appear: `Incorrect terminal type. GenTerm (or MultiCalc, VT52 or VT100) used. Please select terminal type in (S)ystem/Ter(m)inal.`

9. Press the S key and the System menu is displayed.

10. Press the M key to change terminal parameters.

11. Change to your terminal emulation by entering the number followed by Enter.

12. If necessary, change the terminal parameters as described in section 2J.30.10 System/Terminal in this manual.

13. Press any key, e.g. the space bar, when you are told to change emulator.

14. The program will return to the System state.

15. If necessary, change the RS communication parameters as described in section 2J.30.7 System/RS-232C in this manual.

16. Press the Q key.

17. Ready is displayed and the counter is ready for operation.

4.1.6.3 Troubleshooting

If there seems to be communication problems, e.g. characters are displayed but the keyboard does not function, it is probably one of following errors:

Wrong terminal emulator
Example: The counter is configured for GenTerm WT emulation but the PC uses UltroTerm.

Solution: When starting the program, the counter will usually check the emulator in the PC and change its own settings according to this. If this does not work then proceed as follows:

Restart the counter by putting the power switch off and on again. When in the Ready state, press the Esc key (F3 in UltroTerm) one or several times. The counter should respond `Please check terminal type in (S)ystem/Ter(m)inal!`. After this the keyboard will work and you can change the terminal emulation in System/Terminal (press S followed by M). See chapter 2.31 Terminal emulators for further explanations.

Error in communication parameters
Example: The RS-232C communication parameters in the counter differ from those in the PC.

Solution: Set the communication parameters to be the same in the counter and PC. If you are changing the parameters in the counter then restart it as shown above. Go to System/RS-232C (press S followed by R) and set the parameters as described in section 2J.30 System/RS-232C. See section 2.31 Terminal emulators/GenTerm/Changing parameters in GenTerm or 2.31 Terminal emulators/UltroTerm/Changing parameters in UltroTerm for how to change the parameters in GenTerm or UltroTerm emulators, or see the respective manual.

4.1.6.4 Detector usage/shelf usage

In case of a 1, 2 or 3 detector model of MicroBeta, the detector usage must be defined in the system parameters before starting counting. Go to the (S)ystem state and select the (l)evel (T)esting. Then select Detector/shelf (u)sage and choose one of the five detector alternatives.

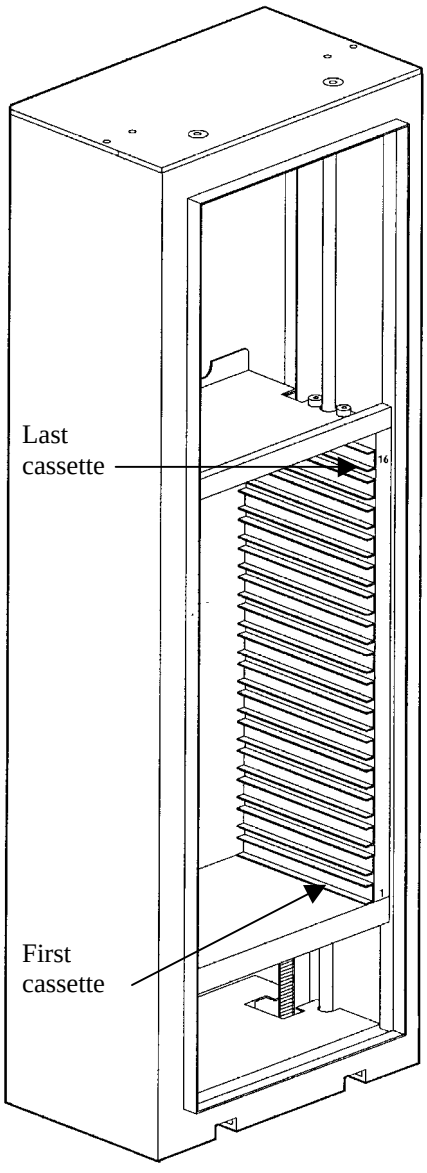
Check also that the shelf usage (1, 16 or 32 shelves) mask type, ParaLux counting and Plate ID reader are set correctly. Here you can also set the selected detector settings to all protocols. Use this feature with care.

4.1.7 Functional check

4.1.7.1 Preparing a functional check

Carry out a functional check of the instrument, including checking the function of the ID reader. To do this proceed as follows:

Prepare four cassettes with labels. On the first cassette use labels 1 - 3 and NORM in the function field (Assay = 1, Protocol = 2, Cassette = 3 and Function code = NORM), on the second 4 - 6 and STD, on the third 7 - 9 and STOP and on the fourth 10 - 12 with the function field empty. See chapter 2.4 for details of coding of a cassette. Insert the first cassette into shelf 1, (see the figure on the right), the last into shelf 16 (32) and the other two somewhere between.



4.1.7.2 Starting the functional check

The ID test is started in the Conveyor state on the test level. To get this go to System by pressing S, select Level by pressing L (this does not appear on the computer display but the figure on the next page shows it). Then select the Test level by pressing T. Go back to the Ready state by pressing Q and there select V (see the example below). Then press T and enter the number of runs. The instrument will run the cassettes once and print the results. After this it only prints ID read errors. Keep the door open and check that the instrument is running alright mechanically.

NOTE! Keep your fingers away from the cassette rack when the door is open!

```
Ready>V
(H)elp              (Q)uit              (O)ff
(C)lear conveyor    Rack (u)p           Rack (d)own          Remove ca(s)s
(N)ext cass         (P)rev cass         Cu(r)rent cass       Ne(x)t pos
(G)oto pos          Si(z)e checkpoint   C(l)ose detector     Op(e)n detector
Next le(v)el        Reset r(a)ck        (I)nit mask          Change (m)ask
Calib (0)-pos       Calib det bloc(k)   (J)ust read IDs      (T)est IDs
(F)ocus ID reader   Print ever(y) ID    Print ra(w) IDs      Print (b)ad IDs
(.)Park det         Shelf (1)..(16)     (*)Calib sensor      Di(s)pIn
Di(s)pOut

Conveyor shelf 1>T

Number or runs 100 ->5

Assay: 1     Prot: 2     Cass: 3    Func: NORM    Shelf: 1
Assay: 4     Prot: 5     Cass: 6    Func: STD     Shelf: 4
Assay: 7     Prot: 8     Cass: 9    Func: STOP    Shelf: 11
Assay: 10    Prot: 11    Cass: 12   Func: NO ID   Shelf: 16
Conveyor stopped

ID test results:    Runs: 5    Errors: 0
                    Read once: 20, twice: 0, thrice: 0
```

The text above should be printed if the test is OK. Check that there are not many occasions when the ID label is read twice or thrice because this shows that the ID reader is performing poorly.

4.1.7.3 Test settings

For checking the performance of the instrument, run the detector efficiency normalization with the normalization standard plate supplied with the instrument. Attach the printout of the measurement to the installation report.

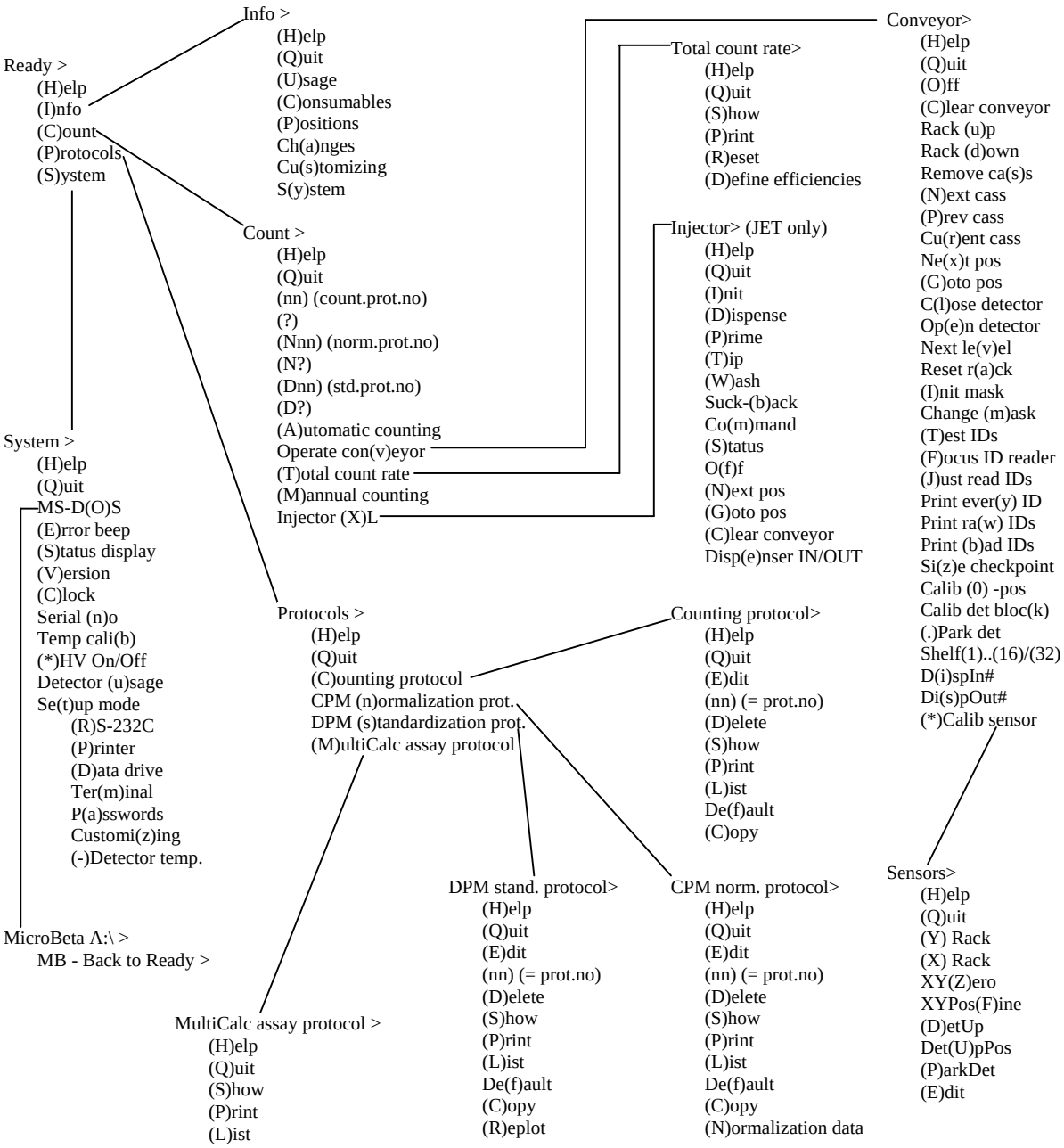
4.1.7.4 System operation showing the TEST level

The figure on the next page is a flow diagram of the whole MicroBeta operating system. It shows how the main control letters (in parentheses) lead to the various functions and how the test functions are related to the normal functions. Compare with the flow diagram in 2J.1.5 showing the functions on the normal level.

4.1.7.5 Leaving the TEST level

To return to the normal level press Q to return to the Ready state, then S to go to the System state. Press L for level selection and N for normal level (not shown). Press Q to return to the Ready state.

Flow chart of MicroBeta commands (service level)



4.1.8 Setting the system parameters

During the installation some system parameters should be checked and set if required. These parameters can be set in the System-state of the instrument program (see chapter 2J.30 System). A checklist of the system parameters is provided in what follows:

4.1.8.1 Terminal emulation

Instrument Manual:

- See chapter 2J.30 System/Ter(m)inal.

Default:

- UltroTerm terminal emulator program.

- 23 lines on screen

Depending on which terminal emulation the user wants to use, check the following:

4.1.8.1.1 GenTerm in WT emulation.

GenTerm is a terminal emulator program supported by MicroBeta. The following parameters must be set if GenTerm is to be used:

(a) Set Terminal emulation to 1=GenTerm.

(b) Set Number of lines on screen at maximum to 24.

Notes:

1: GenTerm V2.B or later is required.

2: See chapter 2.31 Terminal emulators/GenTerm/Installation.

3: See also 4.8.2 and 4.8.5.

4.1.8.1.2 UltroTerm

This is the default terminal emulation and so normally you need not change the terminal emulation settings. However if the terminal PC is some portable model that is not capable of displaying 23 lines then change the Number of lines on screen to the maximum the PC can display.

Notes:

1: UltroTerm V2.00 or later is required.

2: See the sections 2.31 Terminal emulators/UltroTerm/Installation and Terminal emulators/GenTerm/ Troubleshooting.

3: See also 4.8.2 and 4.8.5.

4. 1.8.1.3 MultiCalc
(a) Set Terminal emulation to 3= MultiCalc.

(b) The MultiCalc installation procedure sets other terminal features.

Notes:

1: MultiCalc V1.00 or later is required together with a 1450 communication protocol.

2: See chapter 2.22 MultiCalc and chapter 2.31 Terminal emulators/ GenTerm /Trouble shooting.

3: See also 4.8.2 and 4.8.5.

4.1.8.1.4 Other terminal emulator programs or terminals
MicroBeta can also support other terminal emulator programs and pure terminals that use VT52 or VT100 terminal emulation. If the user wants to utilize one of these then see section 2J.30 System/Ter(m)inal for the possible settings.

Notes:

1: See chapter 2.31 Terminal emulators/VT52 and VT100 compatible terminals about using VT52/VT100 type terminals.

2: See also GenTerm/Troubleshooting in the same chapter.

3: See also 4.8.2 and 4.8.5.

4.1.8.2 Printer connection

Instrument Manual:

- See chapter 2J.30 System/Ter(m)inal.

Default:

- The printer is connected to the terminal PC parallel printer port.

There are two possible ways to connect the printer:

4.1.8.2.1 Printer connected to the instrument RS232C port 3

See the manuals belonging to the printer about how to set up the printer for use with a serial interface.

Go to the Ter(m)inal selection in System-state and set the line Print through terminal to N=No (do not change other lines).

Note: See 4.8.6 for the default RS232C protocol and how to change it.

4.1.8.2.2 Printer connected to the terminal PC parallel/printer port

As this is the default connection there is no need to change system parameters.

Proceed as follows:

(a) Use a standard Centronics-interface cable to connect the printer and the terminal PC.

(b) See the manuals supplied with the printer about how to set up the printer for use with a parallel interface.

4.1.8.3 Printer type

Instrument Manual:

See chapter 2J.30 System/(P)rinter.

Default:

- Epson FX with printing resolution of 12 characters per inch (cpi).

The printer type selection affects only graphical output (e.g. spectrum plots) and the automatic printing resolution setting (cpi). The printing resolution affects the possible line lengths of outputs (10 cpi = 80, 12 cpi=96 and 17 cpi=136 character per line on a standard 8 inch paper). The printer types supported are:

4.1.8.3.1 IBM graphics printer

Select this only if the printer does not support Epson FX emulation but supports IBM graphics printer emulation instead. This mode does not support 12 cpi mode.

4.1.8.3.2 Epson FX

As this is the default mode you need not change printer type if the printer supports this emulation.

4.1.8.4 Clock setting

Instrument Manual: - See 2J.30 System/(C)lock.

Check the date and time and set them to the current values if needed.

4.1.8.5 Data drive

Reference Manual: - See chapter 2J.30 System/(D)ata drive.

Default: - Drive A:.

Select the diskette/hard disk drive and the directory for the MicroBeta result files as described in the Instrument manual. Depending on the terminal emulation set in section 4.1.8.1. you must do the following:

4.1.8.5.1 GenTerm

No need for extra settings.

Note: See chapter 2.31 Terminal emulators/GenTerm/Result files.

4.1.8.5.2 UltroTerm

UltroTerm itself takes care of result file saving.

Note: See chapter 2.31 Terminal emulators/UltroTerm/Result files.

4.1.8.5.3 MultiCalc

MultiCalc itself takes care of result file saving.

Note: See chapter 2.22 MultiCalc and the MultiCalc User Manual chapter on files.

4.1.8.5.4 Other terminal emulator programs or terminals

As commercial terminal emulators differ a lot it is not possible to give any advice which covers all of them.

Note: See the chapter 2.31 Terminal emulators/VT52 or VT100-compatible terminals/Result files.

4.1.8.6 RS-232C-interface protocol

Instrument Manual: - See chapter 2J.30 System/(R)S-232C.

Default (DTR means electronic DTR handshake protocol):

- Port 1=Terminal: 9600 baud, 8 data bits, 2 stop bits, no parity, DTR

- Port 2=External: 9600 baud, 8 data bits, 2 stop bits, no parity, DTR

- Port 3=Printer : 4800 baud, 8 data bits, 2 stop bits, no parity, DTR

Normally these need not be changed. However some situations when they must be are:

(a) Port 1=Terminal: If the terminal PC is going to be used for other purposes during counting (MultiCalc or UltroTerm allow this) then it is recommended to lower the baud rate to 1200 baud or lower to ensure that characters will not be lost.

(b) Port 2=External computer: The interface can depend very much on the external computer used.

4.1.9 Parking the detectors

If the instrument needs to be moved from the place where it was originally installed it is recommended that the detectors should be parked during the time when the instrument is actually being moved. This can be done as follows:

Go to the (S)ystem state and select (L)evel and (T)est. Press Q to return to the Ready state and then O to operate the conveyor. Press . (period). This will move the upper detector block against the lower one so that it cannot shift when the instrument is moved. The following message will be seen on the screen: `Detectors are now parked. Please turn the power off.` When the instrument is restarted the detector blocks will separate again automatically.

4.2 Installation of the Injector System to MicroBeta JET

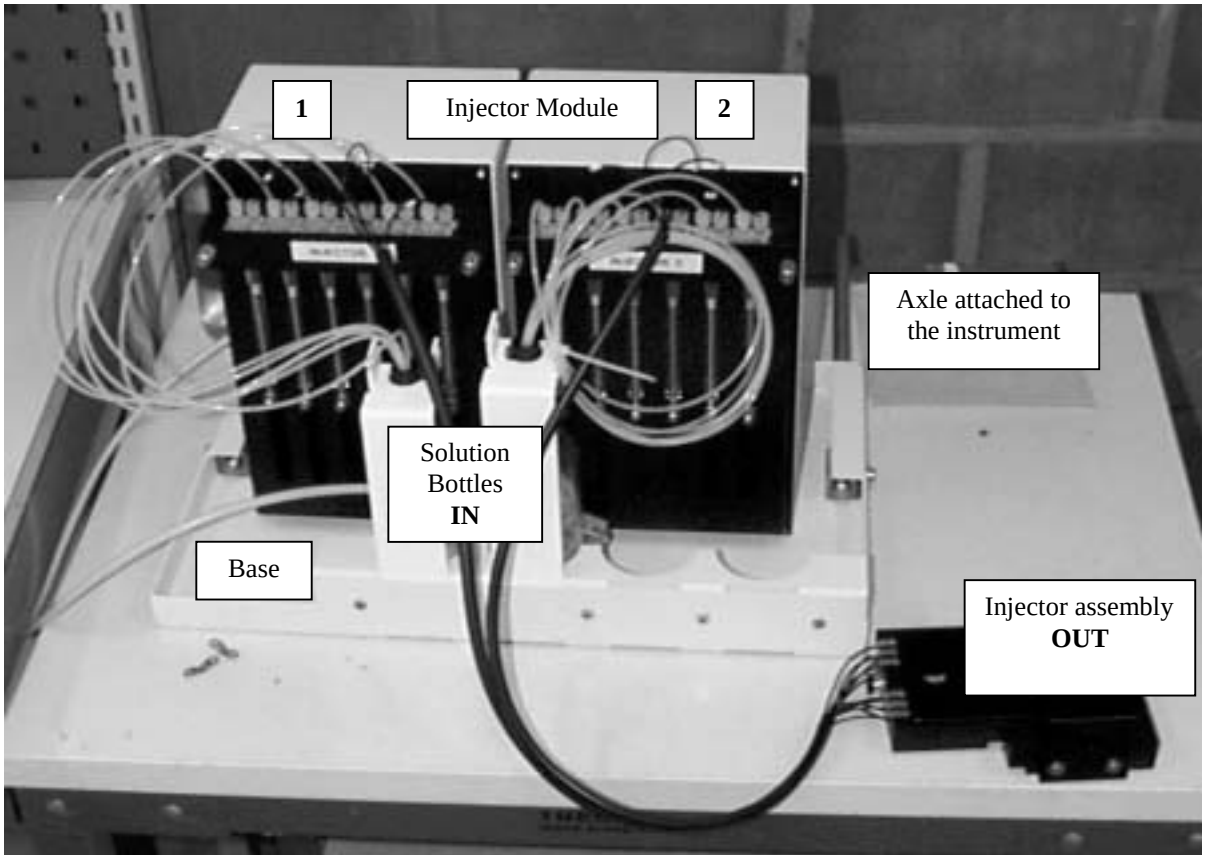
Make a normal MicroBeta installation (mechanical check and check of efficiencies and background). Switch the instrument off and attach the injector system to the instrument:

-attach the injector modules to the base (two screws at the bottom)

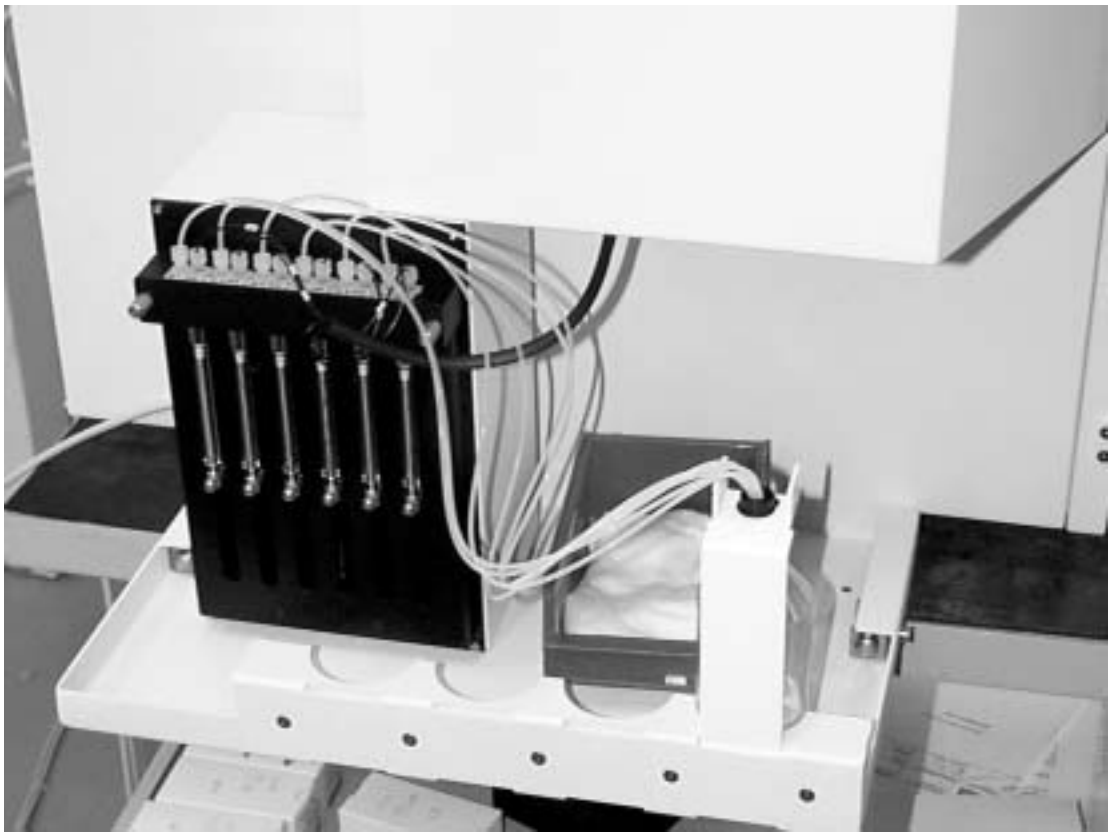
-attach the support pieces for the solution bottles

-attach the transparent tubing of the solution bottles to the IN connectors of the pumps

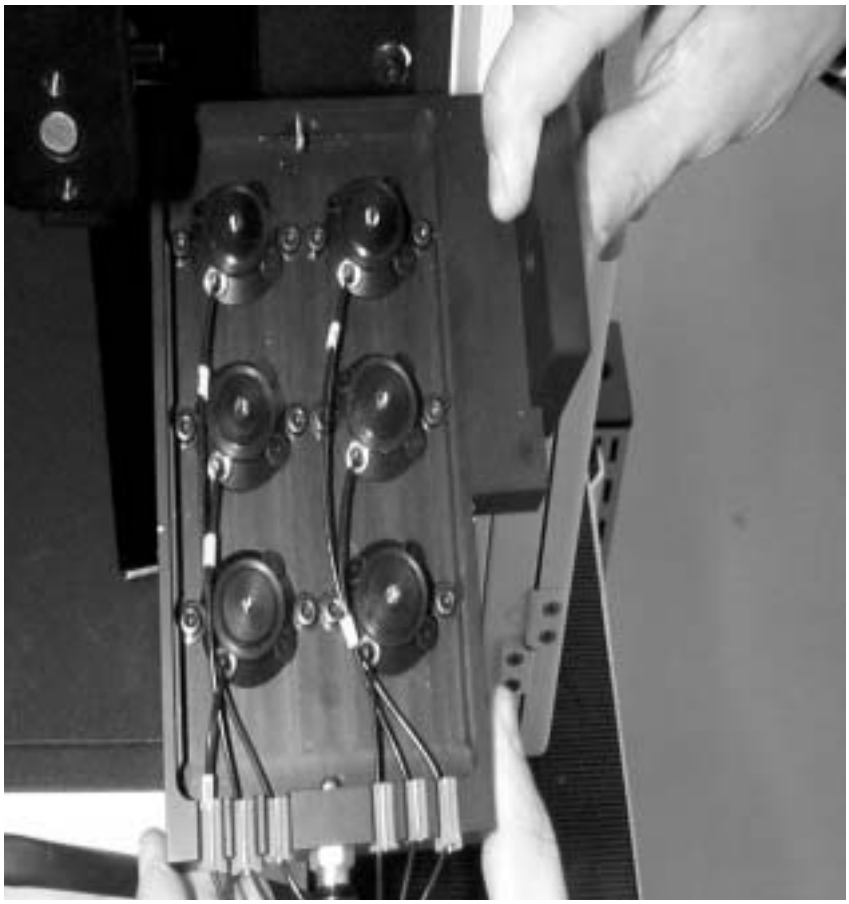
-attach the black injector adapter tube No. 1 to the out connector of pump No. 1 and tube No. 2 to the out connector of pump No. 2 etc. If there is more than one injector module the units are numbered.



-attach the whole injector system to the left side of the MicroBeta JET by sliding the axles into holes in the support pieces attached to the instrument.



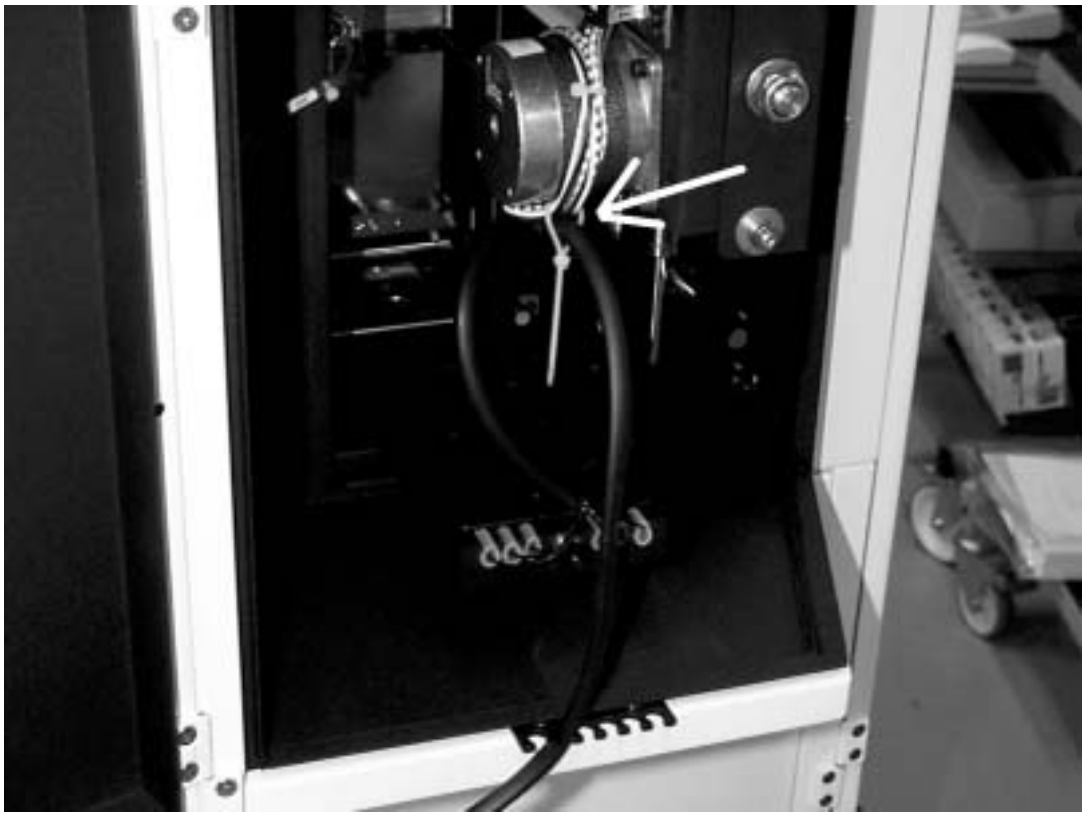
- open the lid of the injector adapter module and check the tubing (see fig below). Systems with two injector modules have a second tube on the left side connector



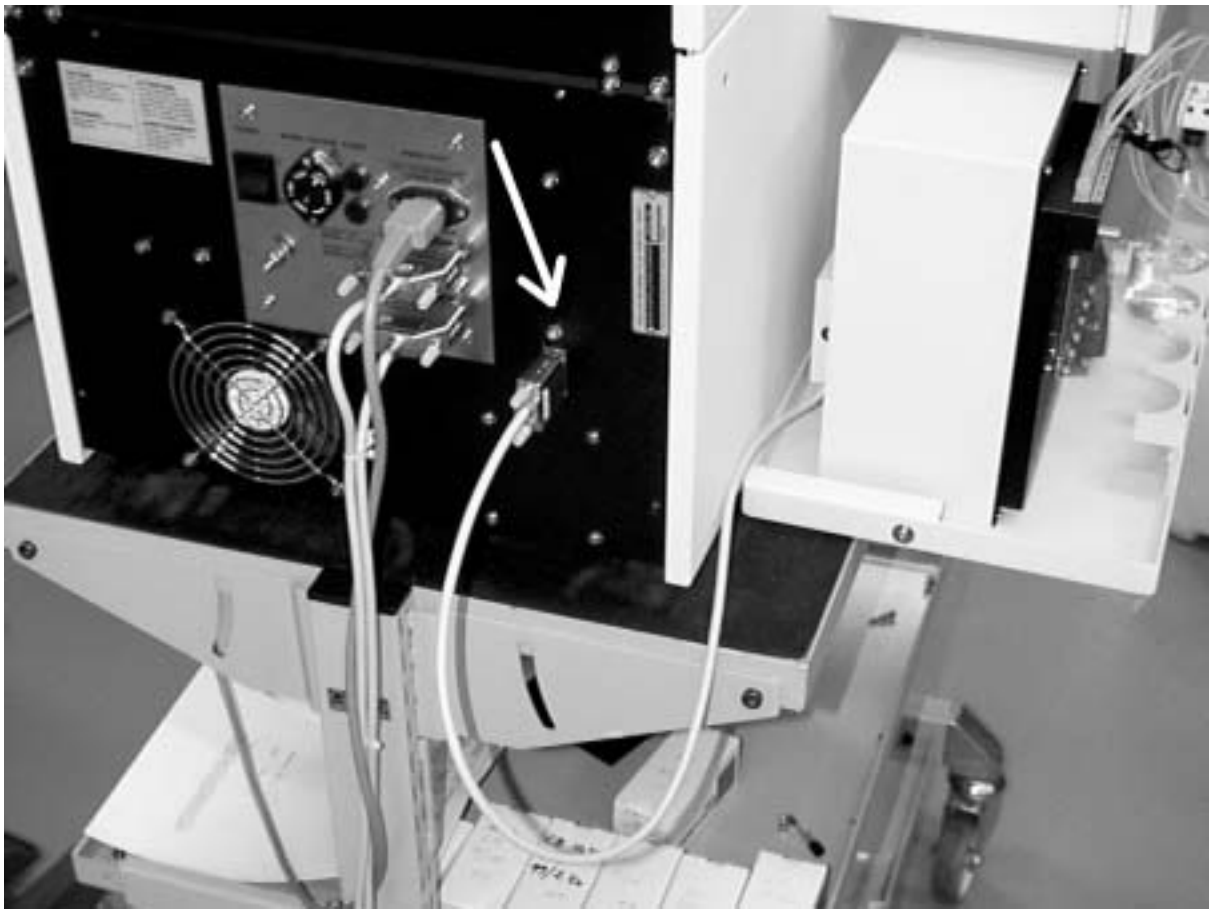
-cut the tie which is attached to the arm for the injector assembly

-attach the injector assembly to the arm (see fig below).

-tie the tubing to the arm motor so the tubing can freely move into the instrument when injecting (see fig below).



-connect the cable from the injector system to the connector at the rear of MicroBeta JET (see fig below)



Boot the system up and check the maintenance section of the User Manual, page 16.

After initializing and priming you should dispense at least once the volume to be used in the assay. The dispensing volumes and alignment to the sample plate can be tested by running a counting protocol. Counting protocol parameters are set for the dispensing speed and volumes for the selected modules.

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